Name(s)
Nitya P. Mehrotra

Project Number
S0515

Project Title
Evaluating the Effectiveness of Inhibitors in Reducing Lipopolysaccharide Induced Tumor Necrosis Factor Alpha Expression

Abstract

Objectives/Goals
Myeloproliferative Neoplasms (MPNs) are malignancies marked by an excess of blood cells. JAK2, Janus Kinase 2, mediates pro-inflammatory cytokines, like Tumor Necrosis Factor Alpha (TNF alpha), through a phosphorylation cascade. Most MPN patients carry the JAK2V617F mutation, which causes constitutive activation of this cascade, leading to increased expression of TNF alpha. Interleukin-10 (IL-10) triggers JAK1, inhibiting TNF alpha expression. Many JAK inhibitors obstruct IL-10, so the purpose of this part of the project was to determine which reduce TNF alpha expression without hindering IL-10.

Methods/Materials
In the experiments with Bone Marrow-Derived Macrophages (BMDMs), TNF alpha was quantified using an Enzyme-Linked Immunosorbent Assay (ELISA), which measures the TNF alpha released into the media from the cells. Nine inhibitors were tested during this part of the experiment. In the experiments with the RAW 264.7 cells, a control group was measured to see the amount of TNF alpha expressed without any drugs, followed by trials using the drugs Curcumin, Trametinib, and N-acetylcysteine. TNF alpha levels were measured using intracellular cytokine staining. A flow cytometer was used to count the number of cells stained due to the expression of TNF alpha.

Results
In the BMDMs, Solcitinib reduced TNF alpha expression by almost 90%, and Momelotinib reduced TNF alpha concentration by around 80%. However, the least effective inhibitor was Ibrutinib, which increased TNF alpha expression by around 40%. In Raw cells, Curcumin was the least successful, reducing concentrations by around 5%, as compared to Trametinib (10%) and N-acetylcysteine (38%).

Conclusions/Discussion
Solcitinib and Trametinib were the most effective. Solcitinib is a JAK2 inhibitor, so it was expected to reduce TNF alpha. Trametinib is a MAPK inhibitor, which inhibits TNF alpha production, so Trametinib was also expected to reduce TNF alpha. Decernotinib and Ibrutinib were the least effective. Ibrutinib is a BTK inhibitor, which is not involved in JAK signaling. Decernotinib is a JAK3 inhibitor that blocks the production of IL-10. Since the IL-10 was already provided, Decernotinib could not block its production. Inflammation is a key symptom of an MPN and as MPNs proliferate, they can develop into leukemia. The inhibitors tested in this project would be used to prevent MPNs from intensifying.

Summary Statement
This project focused on screening the effectiveness of 11 different inhibitors in reducing hematological inflammation to reduce the risk of leukemia in patients with myeloproliferative neoplasms.

Help Received
I conducted my research in the Fleischman Lab in the University of California, Irvine, under the guidance of Professor Angela Fleischman. Additionally, Betty Lai, a graduate student in the lab, helped me conduct test rounds of my Enzyme-Linked Immunosorbent Assays to help me become with the procedure and the