BIOLOGY SUMMER INTERNSHIP

MARLA FORTOUL
SUMMER UNDERGRADUATE PROGRAM AT MD ANDERSON CANCER CENTER

- 10 weeks program
- MD Anderson Cancer Center in Houston, Texas.
- Students will be matched with a faculty mentor in any number of areas of biomedical research where a project will be assigned.
- Participants work alongside the mentor in a lab or clinic on projects designed by faculty to reflect current research.
- Workshops and lectures provide opportunities to connect with faculty, residents, postdoctoral and clinical fellows, and other participants.
- Through the program, students assess goals related to careers in oncology research and patient care.
- Symposium in which participants present talks and posters on their research projects to peers and faculty.
WHY SHOULD YOU GO TO MD ANDERSON?

- **Discover Possible Career Paths**
  - Networking opportunities
  - Learning multiple techniques
  - Shadowing Physicians
  - Oncology
  - PhD
  - MD/PhD

- **Workshops**
  - Simulation Center
  - Speech Elevator
  - Cancer

- **Meet Students from all over the country**
- **Learn about advancements in science**

- **Experience for Resume**
  - Research and clinical

- **Being at MD Anderson**
  - Best Hospital in Cancer Care
  - Groundbreaking research
  - Innovative Clinical Care
  - Clinical Trials
  - Patients travel all over the world to be treated at MD Anderson
DETERMINE THE EFFECT OF IBRUTINIB ON HUMAN T CELLS.

- Department of Stem Cell Transplantation
  - PI: Qing Ma, PhD
  - Dan Li, Sr. Researcher
IBRUTINIB

- Therapy for B-cell malignancies
  - Chronic Lymphocytic Leukemia:
    - It develops slowly, creating defective white blood cells that can’t produce antibodies against infection.
    - The average age of patients at diagnosis is 72.
    - Most Common type of Leukemia

- Effects of Ibrutinib:
  - Inhibits Bruton’s Tyrosine Kinase (BTK)
    - Blocks downstream B cell receptor activation.
  - Investigating the drug’s effect on Inducible T cell Kinase (ITK)
    - Determine the effect on T cell receptor and T cell proliferation.
- Human T-Cell Activation
- Human CD4 T Cell Proliferation
- Human T Cell Counts Inhibited in the Presence of Ibrutinib
- Human CD4 T cell Proliferation in the Presence of Ibrutinib
Ibrutinib Inhibits Human CD4 T Cell Proliferation

Introduction
Ibrutinib is an orally active agent, which works by covalent irreversible binding of Cys-481 in the kinase domain of Bruton’s Tyrosine Kinase (BTK).1 BTK is a non-receptor tyrosine kinase of the Tec-kinase family and is of central importance in B-cell receptor (BCR) signaling.

In addition to BTK inhibition, it has been hypothesized ibrutinib as a target of interleukin-2 inducible kinase (ITK) due to significant homology with BTK. ITK is a T cell dominant member of the Tec-kinase family that drives proximal T-cell receptor (TCR) signaling. ITK is active in T-cell malignancies and has been linked to tumor immune invasion and survival through its critical role in T helper type 2 (Th2) cell differentiation, making it an attractive therapeutic target.2

Objectives
Determine the effect of ibrutinib on human T cells.

Materials and Methods
- PBMCs Isolation
Peripheral blood mononuclear cells (PBMCs) were isolated from donor irradiated PBSCs prepared from whole blood of healthy volunteers donors (Gulf Coast Regional Blood Center, Houston, TX).

- Tissue Culture
PBMCs were cultured in a 95-well V-bottom plate in cell media RPMI (1440, 10% FBS, and 1% P/S) at 0.2 × 10^6 cells well cultured. Cells were cultured for 7 days using 1 µMibrutinib and 1 µM irinotecan or 1 µM ibrutinib and 1 µM dexamethasone for 6 days.

- CFSE Proliferation
A CellTrace CFSE Stained and Diluted in PBS stock (Invitrogen; A11042, 0.5 nM) was added to each well to obtain a final concentration of 1 µM. Cells were incubated for 1 h at 37°C, 5% CO2.

- ibrutinib
An ibrutinib stock concentration of 100 µM was diluted into different concentrations (0.5, 1, 5, and 10 µM) using DMEM. 1 µL of each concentration of ibrutinib was added to PBMCs culture cell.

- Flow Cytometry
Collected T cells were stained with intracellular antibodies: CD3-FITC, CD3-PE, CFSE, PTEN, CD69-PE, and CD25-PE. Samples were acquired on a Canto II Analyzer using Fluorescein-activated cell sorting and analyzed with FlowJo.

Results

Figure 1. Human T-Cell Activation Human PBMCs treated with 100 µM ibrutinib for 6 days. They were stained for expression of CD4, CD8, CD3, CD20, and CD45RA. Results depict (a) SSC and FSC plot with gated lymphocytes, (b) contour plot of expression of gated CD4 and CD8 from live lymphocytes population, (c) CD4 and CD8 gated cells were expanded into four subsets (CD4, central memory, N/NA, TEMRA, terminal differentiated), and (d) marker profile (based on CD45RA and CD45R0) depicting CD4+ (c) and CD8+ (d) cells (e and f) CD4 T cells with Notch1 expression.

Human T-Cell Counts Inhibited in the Presence of ibrutinib

Figure 2. Human T-Cell Proliferation using CFSE assay CD4 T cells stimulated with 1 µM ibrutinib for 6 days. The proliferation index of stimulated and unstimulated T cells was measured with CFSE. The proliferation index of unstimulated and stimulated T cells was measured with CFSE.

Figure 3. Human T-Cell Proliferation using CFSE assay CD4 T cells stimulated with 1 µM ibrutinib for 6 days. The proliferation index of stimulated and unstimulated T cells was measured with CFSE.

Figure 4. Human T-Cell Proliferation in the Presence of ibrutinib

Figure 5. Figure 1. Human T-Cell Activation Human PBMCs treated with 100 µM ibrutinib for 6 days. They were stained for expression of CD4, CD8, CD3, CD20, and CD45RA. Results depict (a) SSC and FSC plot with gated lymphocytes, (b) contour plot of expression of gated CD4 and CD8 from live lymphocytes population, (c) CD4 and CD8 gated cells were expanded into four subsets (CD4, central memory, N/NA, TEMRA, terminal differentiated), and (d) marker profile (based on CD45RA and CD45R0) depicting CD4+ (c) and CD8+ (d) cells (e and f) CD4 T cells with Notch1 expression.

Conclusions
Human T cells can be activated in vitro by using CD3+CD28 beads. CFSE assay measured human CD4 T-cell proliferation showing increased division when stimulated with CD3+CD28 beads. Furthermore, subsets of human T-cells, CD4 and CD8, can be identified using surface markers. Also, subsets of CD4 and CD8 are positive for CD45RA and CD45RO staining markers. These markers allow the identification of naive, central memory, effector memory, and TEMRA CD4 and CD8 human T cells.

It was identified a reduced number of activated human T-cells in vitro as ibrutinib concentration increased until it reached 5 µM which lead to toxicity for the cells resulting in reduced number of bound and increased cell death. CFSE assay measured human CD4 T-cells proliferation in the presence of ibrutinib. It showed an inhibition of CD4 T-cells. The proliferation index of CD4 T-cells decreased as the concentration of ibrutinib increased. It decreased exponentially after 1 µM.

In conclusion, ibrutinib inhibits human T-cell proliferation, in particular, CD4 T cells. The inhibition is dose dependent and starts becoming toxic for the cells at 5 µM.

References

Contact Information
Mark Fatoull
Northwestern University, SURP at MD Anderson Cancer Center
markfatoull@northwestern.edu
APPLICATION REQUIREMENTS

Nova Southeastern registered Halmos College junior or senior for fall 2019 with:
- Science GPA 3.0+ and cumulative GPA 3.0+.
- Successfully completed: Anatomy & Physiology, Medical Terminology, and Statistics.
- Preference to students with upper-level courses.
- Complete application submitted by October 26, 2018 including:
  - Transcript or the CAPP report equivalent
  - Personal Statement
  - 3 Letters of Recommendation
  - Application can be found online at https://cnso.nova.edu/biology/index.html under Summer Internships.
- If qualified, committee interview.

Contact Information:
- Dr. Mark Jaffe, mjaffe@nova.edu
- Dr. Deanne Roopnarine, roopnari@nova.edu
- Dr. Aarti Raja, araja@nova.edu
- Marla Fortoul, mf1612@mynsu.nova.edu