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Analytical Modeling Study of Acute Lymphoblastic Leukemia Treatment with Blinatumomab and Tyrosine Kinase Inhibitors

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ABSTRACT

Acute Lymphoblastic Leukemia (ALL) is a blood-based cancer that accounts for 30% of all pediatric cancer patients and is the second most common type of acute leukemia in adult cancer patients [1]. ALL is an aggressive form of cancer with an average survival rate of only 5 years, suggesting that novel treatment options are needed. Treatment methods such as chemotherapy are often unsuccessful at treating ALL, as they are not specific and can lead to relapses [2]. Two therapeutics of interest are bispecific T cell engagers (biTEs) and tyrosine kinase inhibitors (TKIs), both of which address the specificity issues that arise with chemotherapy. BiTEs, also known as bispecific antibodies, are comprised of two monoclonal antibodies held together by a peptide linker. One end of the biTE binds to CD3+ T cells and the other end binds to CD19+ B cells. These can be used in cancer therapeutics to recruit immune cells to the site of cancer cells. TKIs work to inhibit their respective tyrosine from phosphorylating tyrosine residues and inhibit pathways downstream of the phosphorylation in the malignant B cells. There are ongoing clinical trials on treatment of Philadelphia Chromosome positive (Ph+) B cell ALL with the combination of TKIs and the biTE blinatumomab. One clinical trial investigated treating the ALL with blinatumomab and ponatinib and the other investigated blinatumomab with dasatinib. The rationale behind this combination is to inhibit kinase activity in malignant B cells while also promoting an immune response through activating T cell receptors and recruiting T cells [3]. However, previous studies have found that when used in combination, TKIs involved with the Src family kinases and biTEs have an antagonistic effect on cancer treatment [4, 5]. Through *in vitro* experimentation and mathematical modeling, it is possible to explore the antagonism mechanism of TKIs and biTE combination therapy.

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Figure 1: Independent

Figure 2: Gabora et al 2019

Figure 3: Independent

Figure 4: Bass et al 2017

Figure 5-10: Independent with references to common knowledge and literature as noted

Figure 11: Farnaz Naeemikia and Josh Reynolds for showing me how to run and collect data for the experiment

Figure 12-13: Farnaz Naeemikia and myself curve fitting data from figure 10 experiment

Figure 14-15: Independent

Figure 16: Farnaz Naeemikia for running the optimization assay and imaging the blot and Mikayla Shaffer for analyzing data with me

Figure 17-20: Farnaz Naeemikia for helping me run a successful replicate with me and Mikayla Shaffer for analyzing data with me

Chapter 1

Introduction

1.1 Motivation and Background

Cancer is a disease that affects millions of people worldwide and leads to 600,000 deaths in the U.S. each year alone [1]. Acute Lymphoblastic Leukemia (ALL) is the second most common type of cancer and stems from mutations in a patient's bone marrow, blood, and extramedullary sites in bone. ALL affects mostly children, with only 20% of total cases in adults [6]. A major issue with current treatment options like chemotherapy, is the lack of specificity in targeting cancer cells. Patients treated with chemotherapy have strong side effects with a low rate of long-term success [7]. Because nonspecific medications are not successful at targeting ALL, other treatment options like immune cell-mediated therapy have become more relevant because they could potentially target cancer cells, more effectively.

The purpose of this thesis is to create a stochastic model of T lymphocytes and malignant B cells dynamics during combination therapy with bispecific T cell engagers (biTEs) and Tyrosine Kinase Inhibitors (TKI). BiTEs fall under the classification of bispecific antibodies, which are created from two monoclonal antibodies linked together by a flexible peptide chain. BiTEs are designed so that one antibody side binds to the cancer cell through CD19+ cells, while the other side binds to CD3+ lymphocytes to recruit T cells to attack the malignant B cells [8]. TKIs are commonly used in the treatment of cancer and for the purposes of this thesis, I will mainly focus on comparing TKIs involved with the Src family kinases and in inhibiting the phosphorylation of lymphocyte-specific protein tyrosine kinase (LCK) such as dasatinib and

ponatinib to non-Src TKIs such as imatinib and nilotinib [4, 5]. Previous research suggested positive effects of Src TKIs when used in combination with blinatumomab for the treatment of Ph+ ALL [3]. However, recent studies have demonstrated antagonistic effects when using the combination of Src TKIs with blinatumomab, and their findings suggest this combination leads to a decreased efficacy in the biTE [9]. This thesis focuses on TKIs involved in inhibiting the Src family kinases and TKIs involved in inhibiting other pathways. Src specific TKIs used in this thesis include dasatinib and ponatinib, while non-Src TKIs include nilotinib and imatinib.

1.1.1 Bispecific Antibodies

Bispecific antibodies are engineered to bind to two different antigens or two different epitopes on the same antigen. For example, Blinatumomab is a BiTE antibody (bispecific T-cell engager antibody) that has Fv fragments, or in other words fusion molecules, from anti-CD3 and anti-CD19 arms joined [6, 10]. They are joined by a nonimmunogenic linker. Blinatumomab brings together cytotoxic CD3+ T cells and CD19+ B cells and results in T cell proliferation as well as B-cell apoptosis [4, 11].

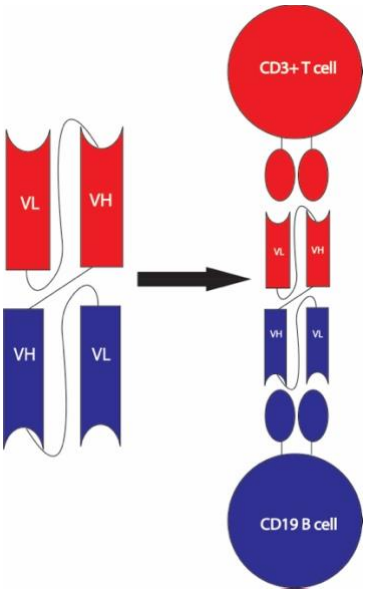


Figure 1: Bispecific antibody schematic.

1.1.2 Tyrosine Kinase Inhibitors

Tyrosine kinase inhibitors inhibit their corresponding kinases from phosphorylating tyrosine residues of their substrates and then block the activation of downstream signaling pathways [12]. For example, one TKI called dasatinib inhibits the Src family kinases [13]. LCK is a member of Src family that plays a pivotal role in T-cell receptor signaling.

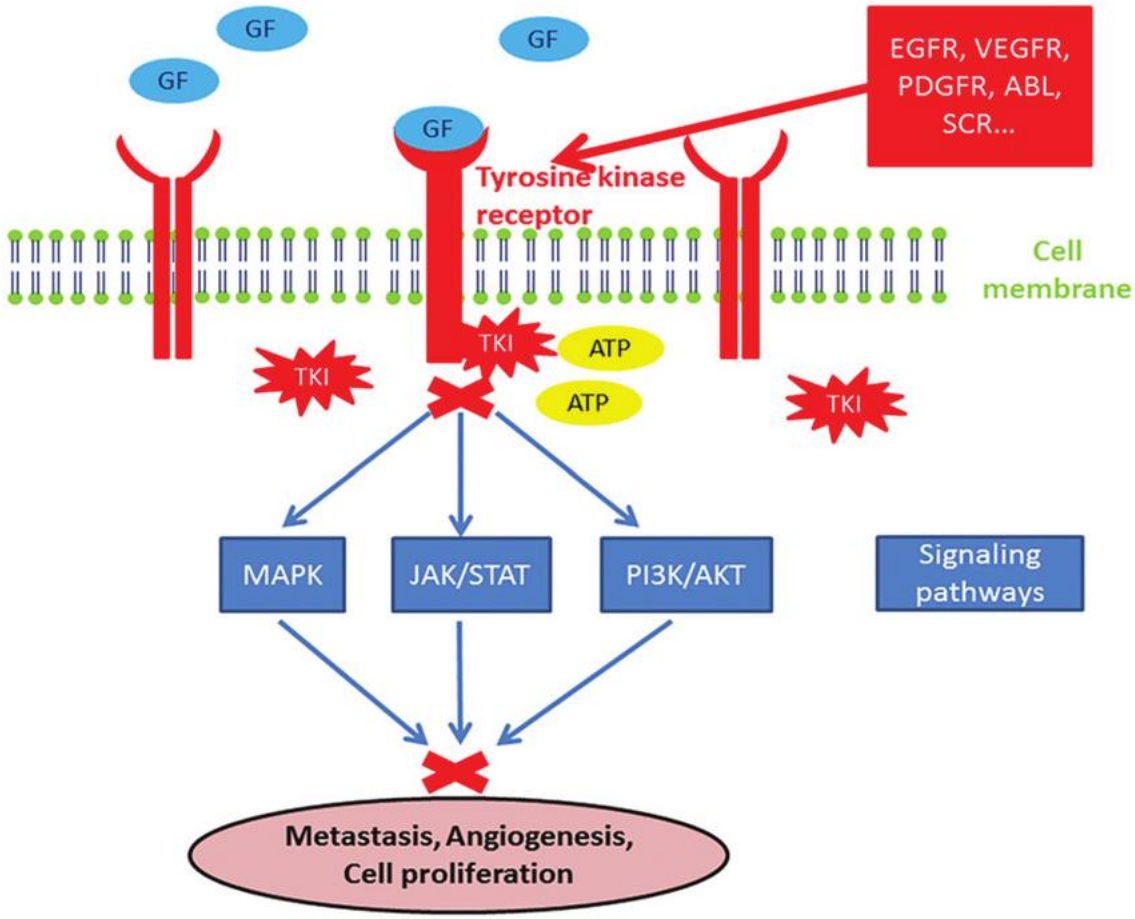


Figure 2: TKI schematic from Gabora et al. 2019 **[14]**.

1.1.3 Combination Therapy of BiTEs and TKIs

Acute Lymphoblastic Leukemia (ALL) is a blood-based cancer that accounts for 30% of all pediatric cancer patients and is the second most common acute leukemia in adult patients. ALL is an aggressive form of cancer, and the average survival rate is only 5 years. There is an ongoing clinical trial on treatment of ALL with the combination of TKI and Blinatumomab. Previous studies have found that when used in combination, biTEs and TKIs have an

antagonistic effect on the treatment of cancer [9, 11, 13]. In this research, we purpose to develop a mathematical model to test two biological plausible hypotheses regarding the combination therapy with TKIs and biTEs. we will test whether the decrease in T cells proliferation is due to an antagonistic effect on the LCK activity or depletion of the malignant B cells.

1.2 Objectives

Through *in vitro* experiments, cell culture assays, and flow cytometry, we plan to find values related to the rates of growth, division, and decay of our T cells and B cells to feed into the model, estimate unknown parameters and find the key determinants of biTEs success of treatment. We will be able to determine the growth rate of T cells, death rate of T cells, growth rate of ALL cells, death rate of ALL cells through *in vitro* experiments and estimate other values of interest through the mathematical model. With Michaelis Mentin enzyme kinetics as a framework, we will be able to create a set of ordinary differential equations to model our biological systems. The experiments will serve to validate our model in order to check the known parameters and then determine the unknown parameters.

Once our model is fully developed, we will be able to further validate it by performing *in vitro* experiments with CD3+ and CD19+ interactions in the presence of biTEs and TKIs in combination and separate, respectively. Through these *in vitro* experiments we intend to test our research question of whether the T cell deficit we see is due to a lack of phosphorylated LCK, a member of Src family kinase or the depletion of ALL cells. With this information, we will be able to better understand the data of clinical trials that have used TKIs and biTE in combination to treat ALL. We will be able to see if this combination leads to a more effective treatment or if

it causes an off-target effect, as we believe biTEs interfere with the TKIs ability to inhibit Src family kinase activity.

1.3 Thesis Organization

This thesis contains five total chapters. Chapter 1 explains the problem along with relevant background and motivation for the study. Chapter 2 describes the materials and methods used to perform each experiment contained in the thesis. Chapter 3 focuses on the computational model designed for the thesis to model the interaction between T-cells and malignant B-cells in a no treatment condition as well as treatment conditions. Chapter 4 uses experimental data to show the mechanism that can possibly rescue the antagonistic effect of Src TKIs on LCK phosphorylation inhibition through the pSTAT5 pathway.

Chapter 2

Materials & Methods

2.1 Cell Culture

T lymphocyte Jurkat cells, Philadelphia chromosome (Ph1)-positive acute leukemia BV-173 cells, and peripheral blood mononuclear cells (PBMCs) were utilized for the experiments in this thesis. All cells were cultured in tissue treated polystyrene petri dishes at 37^{o}C and 5% CO_2 in an incubator with RPMI 1640 media with 1% penicillin streptomycin and 10% FBS.

2.1.1 Jurkat Cell Culture Protocol

Jurkat cells were cultured in RPMI 1640 media solution with 1% penicillin streptomycin and 10% fetal bovine serum (FBS). Between 1-2x10^6 cells were seeded into the petri dishes with 15 mL of RPMI that was previously warmed to 37^{o}C. The cells were checked daily and grown in the incubator until confluent. Once confluent, the contents of the petri dish were pipetted into a 15mL centrifuge tube and centrifuged at 500g for 5 minutes. Post centrifugation, the supernatant was aspirated off and the cells were resuspended in media to be counted and passaged or seeded for an experiment.

2.1.2 BV1-73 Cell Culture Protocol

BV-173 cells were cultured in RPMI 1640 media solution with 1% penicillin streptomycin and 10% FBS. An identical protocol to Jurkat was used to culture the cells.

2.1.3 PBMC Cell Culture Protocol

PBMC cells were cultured in Dulbecco's Modified Eagle Medium solution with 1% penicillin streptomycin and 10% FBS. The cells were grown with a similar protocol to the Jurkat cells and BV-173 cells and were centrifuged at 500g for 5 minutes to prepare the cells for passaging or seeding in an experiment.

2.2 Cell Counting

Cells were counted by hand using a hemocytometer. After aspirating the supernatant from the tube of centrifuged cells, the cells were resuspended in 1 mL of their perspective media. Then, 10 uL of the mixed solution was ejected into a 1.5 mL Eppendorf tube and 10 uL of trypan blue was mixed into the tube as well. The trypan blue stained dead cells blue, while live cells remained bright under the microscope. Then, 10uL of this solution was ejected into the hemocytometer and the cells were counted in the four corner boxes and center box. The result was divided by 5 (to obtain the average cells per $1mm^2$ square), multiplied by 2 (to account for the dilution factor) and then multiplied by 10^4 (to account for the total cells in the original sample). The result from this gave the total cells in the newly suspended 1mL solution of cells.

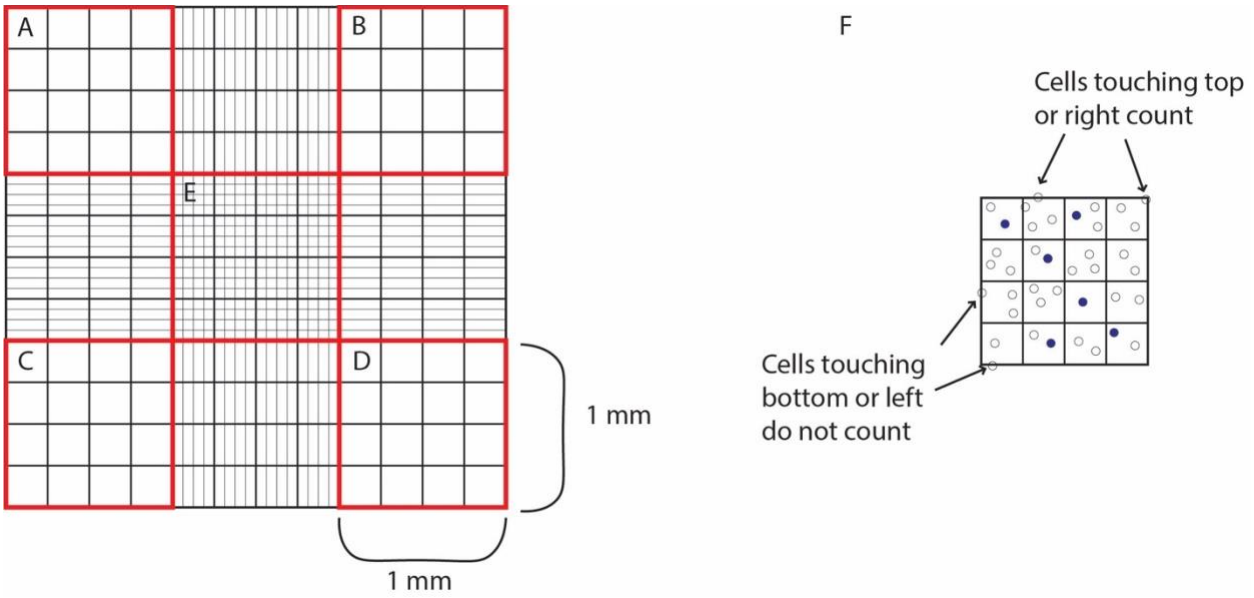


Figure 3: Cell counting with a hemacytometer.

Cells are counted with a hemacytometer by counting all cells in the 1 mm x 1 mm squares labeled A-E. Cells touching the top or right of the square are counted while cells touching the bottom or left of the square are not. Trypan blue stains dead cells blue, so ant blue cells as seen in F are not counted. After counting the total cells, the number is divided by 5, then multiplied by the dilution factor and 10,000 cells/mL. This gives the number of cells per milliliter of sample.

2.3 Cell Staining

Cell staining was used to visualize T cells and B cells as well as identify other cell components in the experiments. Typically, 1mL of cells were added to Eppendorf tubes and centrifuged at 500 rcf for 5 min at 4ºC. The old media was then aspirated off the cells and blocking buffer was added for 10 minutes. All cells were blocked with 10uL Fc block added to lmL of 3% BSA in 1XPBS unless otherwise specified. After 10 minutes, all tubes were centrifuged at 500 rcf for 5 min at 4ºC. Then, 2uL of primary antibodies were added to 200uL of 3% BSA in 1XPBS and added to the cells. The solution was then incubated covered with foil for 20 minutes at 4ºC and then brought to room temperature for 15 minutes. The solution was

washed with 3 times with 1XPBS. After each wash, the cells were centrifuged, and the supernatant was aspirated off.

2.4 Flow Cytometry

Following the cell staining assay, 100uL of 3% BSA was added to each tube to prepare for flow cytometry. BD Acuri was used to run the flow cytometry run with limited and 10uL volume, fluidics was set to “medium”, the sample was loaded into the probe, and the “run” button was clicked.

For flow cytometry, a negative control with no staining was added first and then the stained conditions were loaded after to compare mean fluorescent intensity of stained cells. A line could be drawn onto the graph to compare control to experimental conditions and see differences in stained versus no stained populations. For live/dead analysis, a circle could be drawn around cells in the upper right corner of the FSCA versus SSCA plot and the percentage of live cells could be quantified, as live cell populations are larger in size compared to dead cells.

2.5 Drug Treatment Cell Count Assay

A drug treatment cell count assay was performed to test the effects of combination therapy on T cell and B cell growth. PBMC and BV173 cells were used in the experiment. In this experiment drug conditions included: no treatment, blinatumomab, blinatumomab + dasatinib,

and blinatumomab + nilotinib. In total 5,000 PBMC cells were added to 10,000 BV173 cells on day 0 and cocultured. The treatment concentrations for dasatinib, nilotinib, and blinatumomab were 10nM, 130nM, and 0.1 ng/mL respectively. Each condition was treated with the drugs of interest and incubated in RPMI with 1% penicillin streptomycin and 10% FBS in the cell incubator. Starting on day 0 a sample of the cells were stained for CD3 and CD19 and then quantified using flow cytometry. Cell counts were then taken each day for 6 days total with each treatment condition.

2.6 Drug Treatment Western Blot Assay

2.6.1 Cell Lysis Extraction

A drug treatment western blot assay was performed with the addition of interleukin 2 (IL2), interleukin 7 (IL7) and/or interleukin 15 (IL15) to test the effect of IL2 on the TKI conditions. The conditions tested were with an unstimulated negative control of only Jurkat cells as well as cells stimulated with CD3/CD28 magnetic beads in an untreated condition as well as treated with ponatinib, dasatinib, nilotinib, and imatinib. These six conditions were replicated and treated with interleukin as well to form 12 total conditions.

Each condition used 2 million Jurkat cells that were placed in serum starved media for four- or sixteen-hours pretreatment. In total 24 million cells were left with no stimulation, while the remaining 20 million were stimulated with CD3/CD28 magnetic beads after serum starving. The beads were prepared by collecting 10 uL of beads to an Eppendorf tube with RPMI and vortexing for a few seconds. The tube was then placed on a magnet for 5 minutes and the RPMI

was aspirated off. The washing process was then repeated two times and the final beads were diluted in 550uL of RPMI.

After serum starving, 10uL of beads per 2 million cells were added for the conditions that required stimulation and incubated for 2 hours and then drugs were added for 2 more hours. After stimulation, each condition was treated with the drugs and/ or IL2, IL7, IL15, or ILC and incubated for 2 hours. The treatment concentrations for ponatinib, dasatinib, nilotinib, imatinib, and IL2 were 40nM 10nM, 130nM, 450nM, and 100ng/mL respectively.

During treatment, a cell lysis buffer was prepared with 100uL lysis buffer, 900uL PBS, 2uL PMSF protease inhibitor, and 4uL PMSFA. After the two hours of treatment, each condition was centrifuges in Eppendorf tubes for 5 min at 500 g, then the supernatant was aspirated off and 50uL of the buffer was added to each cell pellet. The tubes were incubated in 4ºC for 30 minutes, vortexing every 10 minutes to ensure proper lysing. After 30 minutes, each tube was centrifuged at 1600 rcf for 15 min, and the supernatant was aspirated off.

2.6.2 Bicinchoninic Acid (BCA) Protein Assay

To see the concentrations of the lysis, a Bicinchoninic acid (BCA) protein assay was performed using a Thermo Fisher BCA kit. In the kit 9 standards were prepared and labeled A-F. In vial A, 300uL of stock was added. In vial B, 375uL stock was mixed with 125uL PBS. In vial C 325uL of stock was added with 325uL PBS. In vial D 175uL of vial B was added with 175uL PBS. In vial E 325uL of vial C was added with 325uL PBS. In vial F 325uL of vial E was added with 325uL PBS. In vial G 325uL of vial F was added with 325uL PBS. In vial H 100uL of vial G was added with 400uL PBS. In vial I 100uL PBS was added.

To prep each standard and experimental sample, 5uL of the sample was added to a 96 well plate with 100uL of the working reagent (WR). This was repeated for a total of 2 replicates. The plate was incubated at 37 degrees Celsius for 30 minutes. All samples were cooled to room temperature and read using Envision manager and a 562nm wave absorbance.

The software read the plate and output a csv file. To standardize all samples the reading from the blank was subtracted from all samples and then standard curves were created in Microsoft Excel. Using the standard curve, the experimental points were fit, and the protein concentrations were determined.

2.6.3 Western Blot

To prepare for the western blot, 1x running buffer was made in advance with 50mL running liquid in 950mL milli-Q water and 1x transfer buffer was made with 50mL transfer liquid in 950mL milli-Q water.

Calculations for each experimental sample were made using the BCA data to result in approximately 10ug protein in 20uL of sample buffer and sample volume. A 2x lysis buffer was made with 500uL 1xPBS and 500uL NuPAGE LDS sample buffer. The buffer was added to each sample for a total volume of 40uL. Each tube was transferred to a 80ºC bath for 10 minutes to denature the protein. Using a 4-12% NuPAGE gel, the strip and comb were gently removed and put in the containment system with the comb side up. Running buffer was added to cover the gel and each well was washed using a pipette. The ladder was loaded to the first cell with a volume of 15uL and the rest of the samples were loaded in in volumed of 15uL each. The gel was run in

the 4ºC fridge, the lid was attached, and electrodes were placed on the lid. A 150V was applied with 3.00A and 300W for 2 hours.

After 2 hours, half of the liquid was removed from the device and 3 absorbent pads were placed in transfer buffer in an empty pipette tip container. A 0.45 nitrocellulose membrane filter was placed in the transfer buffer with the absorbent pads. The device was opened, and the gel was carefully cut out and placed on top of a sponge and filter in a western blot transfer box. The membrane was placed on top of the gel to ensure proper transfer and all bubbles were rolled out and sponges were added on top until a tight seal could be made in the box. The box was then loaded into the device used for the initial western blot run and the transfer buffer was added to submerge the device. In the 4ºC, the blot ran for 2 hours with 150V, 3.00A and 300W applied. The blot was then removed, and tweezers were used to delicately remove the membrane. Then, 5mL of 5% BSA in TBST was added to the membrane in the empty pipette tip box and left covered on the shaker for 1 hour.

While waiting for the blocking, 2uL of primary antibodies were added to 5mL of the 5% BSA TBST solution. This primary antibody solution was added to the membrane and incubated on the shaker in the 4ºC fridge overnight. The next morning, the membrane was washed 3 times with TBST for 15 minutes each time. Secondary antibodies were added for 1 hour at room temperature and then washed 3 times with tween 20 (TBST) for 15 minutes to wash the nitrocellulose membrane. The chemiluminescent detection solution was made by adding 1mL of Part A to 1mL of Part B. After adding the detection solution to the blot for 1 minute, the blot was imaged. After imaging the blot, it was stripped for 30 minutes in stripping buffer, washed with dH2O for 15 minutes, and washed with TBST for 15 minutes. Then the blocking, staining, and imaging protocol were repeated for all antibody conditions.

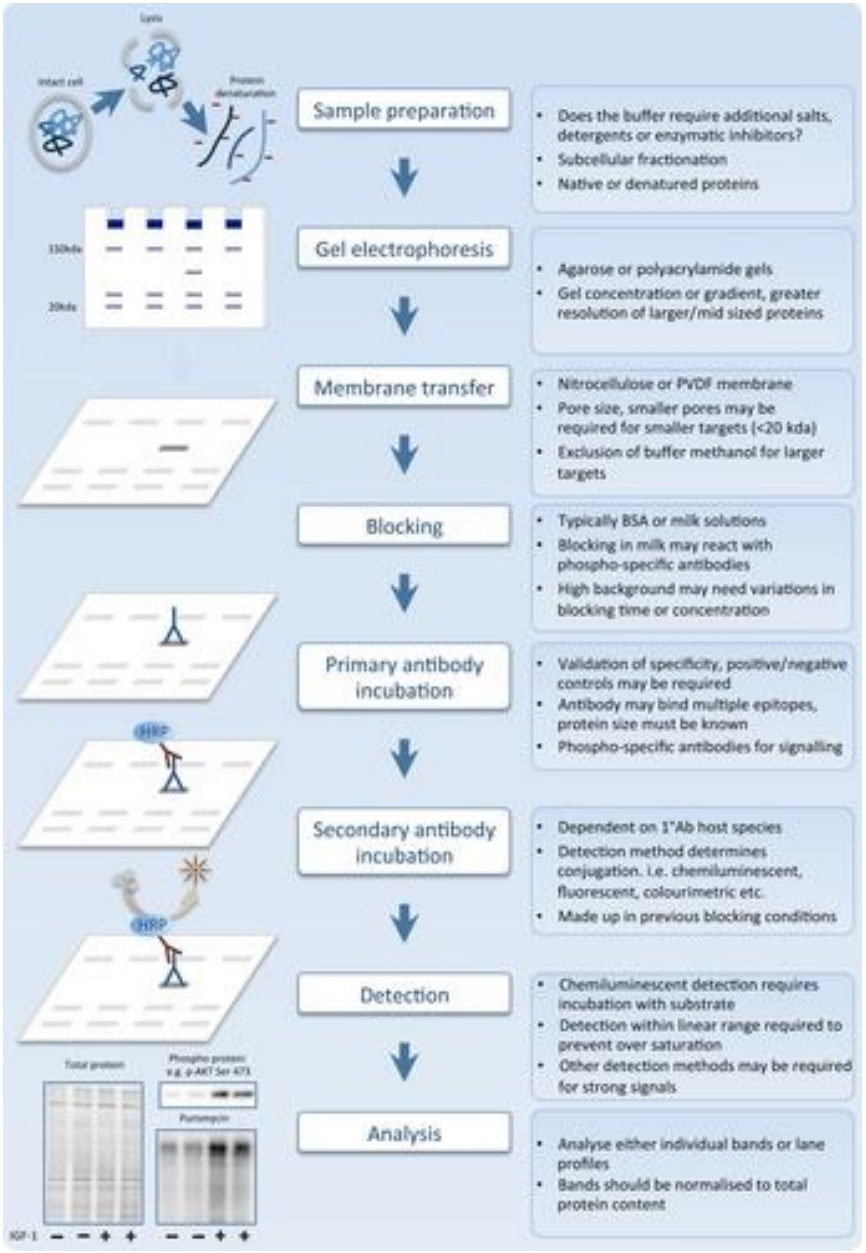


Figure 4: Western blot schematic **[15]**

Figure 4 shows an overview of performing a western blot, similar to the process describes above in the text.

2.7 Statistical Analysis

Statistical analysis is described in the individual sections for the specific experimental data collected.

Chapter 3

Modeling T cell and B cell Dynamics Without and With Treatment

3.1 Michaelis-Menten Enzyme Kinetics

Michaelis-Menten enzyme kinetics are often used to explore the reactions that occur between and enzyme and substrate in a reaction where the enzyme and substrate form a complex and regenerate into the original enzyme and form a product [16]. A typical equation of Michaelis-Menten kinetics is shown below:

$$E + S \overset{K_1}{\rightarrow} ES \overset{K_2}{\rightarrow} E + P \qquad \text{Equation 1}$$

$$E + P \overset{K_3}{\rightarrow} ES \overset{K_4}{\rightarrow} E + S \qquad \text{Equation 2}$$

In this equation E represents the enzyme, S represents the substrate, ES represents the enzyme substrate complex, and P represents the product. There are various rates associated with the forwards and backwards reactions, where K_1 is the rate of complex formation from reactants E and S, K_2 is the rate of product formation from the complex, K_3 is the rate of the reverse

catalysis reaction, and K_4 is the rate of complex dissociation. There are a few assumptions when using Michaelis-Menten such as a closed system, fast binding, slower product formation, starting product concentration of zero, a greater concentration of substrate in comparison to enzyme, and enzyme only existing in its original form and the complexed form [17, 18, 19]. Michaelis-Menten kinetics have been used for a variety of biomedical applications such as modeling cell population dynamics over time [20].

The mechanism of action of biTEs acts to bring CD3+ T cells together with CD19 B cells to form a complex and stimulate T cell proliferation to target malignant B cells circulating in a patient's system. Because of this, a system based on Michaelis Menten kinetics was developed to predict cell outcomes with the introduction of biTEs.

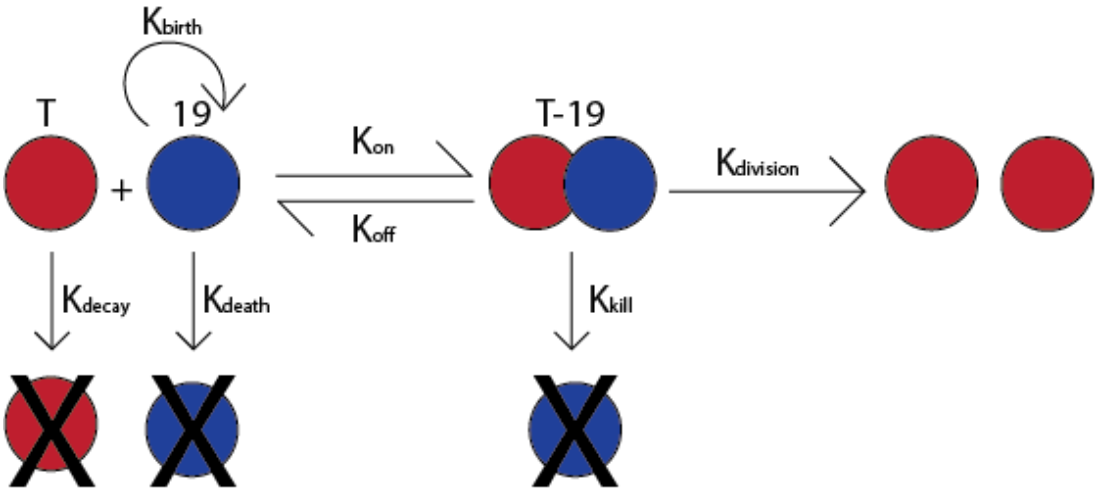


Figure 5: T Cell CD19 Cell Interaction with Introduction of biTE.

When a biTE is introduced to the system, a CD3+ T cell (T) and CD19 B cell (19) are joined together to form a ternary complex which can result in CD19 apoptosis and T cell proliferation. Before linked, T cells and CD19 cells have decay rates and CD19 cells have birth rates.

Through developing a schematic model of potential outcomes with biTE intervention, a series of differential equations were developed to better understand the effects on T cells, CD19 Cells, and the T cell- CD19 complex as shown below:

$$\frac{dT}{dt} = K_{division}[T19] - K_{decay}[T] - K_{on}[T][19] + K_{off}[T19] \quad \text{Equation 3}$$

$$\frac{d19}{dt} = -K_{on}[T][19] + K_{off}[T19] + K_{birth}[19] - K_{death}[19] - K_{kill}[19] \quad \text{Equation 4}$$

$$\frac{dT19}{dt} = K_{on}[T][19] - K_{off}[T19] - K_{division}[T19] \quad \text{Equation 5}$$

In this system of ordinary differential equations, there are various rates associated with each outcome. The equation related to T cell change over time is related to the $K_{division}$ or proliferation rate of T cells post complex, as well as the decay rate of T cells and the K_{on} and K_{off} rates of complex formation and disassociation. The equation related to CD19 B cell chance over time is related to the K_{on} and K_{off} rates of complex formation and disassociation as well as the K_{birth} birth rate of CD19 cells, K_{death} decay rate of B cells and K_{kill} rate of B cells killed post complex. The equation related to the T-19 complex relates to the K_{on} and K_{off} rates of complex formation and disassociation as well as the $K_{division}$ proliferation rate of T cells post complex disassociation.

3.2 MATLAB Model Development

Using the equations developed through the model of biTE mechanism of action, a computational model was developed in MATLAB. A function was created to encompass the rates of T cell, B cell, and complex formation.

```
function[output]= odefun(~, y, coeff)

% ode function 1
dTdt=coeff(6)*y(3)-coeff(3)*y(1)-coeff(1)*y(1)*y(2)+coeff(2)*y(3);

% ode function 2
dCD19dt=-coeff(1)*y(1)*y(2)+coeff(2)*y(3)+coeff(4)*y(2)-coeff(7)*y(2);

% ode function 3
dsdt=coeff(1)*y(1)*y(2)-coeff(2)*y(3)-coeff(6)*y(3);

output=[dTdt; dCD19dt; dsdt];

end
```

Figure 6: MATLAB function for modeling T cell B cell and complex rates.

In the figure, the MATLAB code is shown for modeling the three differential equations of interest. Each coeff() corresponds to a rate constant K as previously described and y(1), y(2), and y(3) correspond to the concentrations of T cells, B cells, and complexes respectively.

With the model, a timespan could be input by a user as well as estimates for each K rate constant, and then plotted into a graph with a curve for T cell, B cell, and complex concentrations over time. A sample run through of the model is demonstrated in the below figure.

A

```
clc
clear

timespan= [0 100];
IC= [1 1 0];
% IC in  order of T-cell CD19 T-CD19 complex

coeff= [0.7 0.01 0.05 0.07 0.1 0.2 0.001];
% coefficients in order of Kon Koff Kd1 Kb Kk KD (divide) kd2

options=[];
[t,y]= ode45(@odefun, timespan, IC, options, coeff);

plot(t,y(:,1), "b", t,y(:,2), "r", t,y(:,3), "g")
title("Concentration v time")
xlabel("Time (s)")
ylabel("Concentration")
legend("T-cell", "CD19 cells", "T-CD19 Complex")
```

B

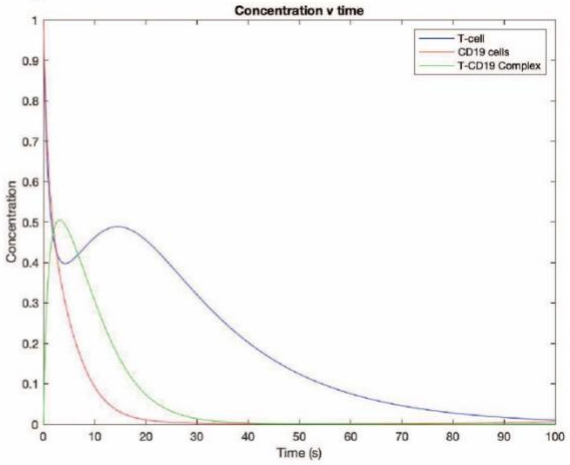


Figure 7: Sample script run through and output.

A user can input initial conditions for complex, T cell, and B cell count as well as put in K values of interest to study. Then the script feeds these numbers into the ODE function and plots the results.

3.3 MATLAB Model Order of Magnitude Study

With the MATALB model created, it was of interest to determine how sensitive each K value was in the model. Performing a sensitivity analysis would help show which K values would be the most important and lead to the largest changes in the shape of the growth curves. By determining the most and least sensitive variables, it would show which variables were most important to approximate with biological values and *in vitro* experimental values. This would also show which K values were less important in determining the shape of the outputted growth curves. By knowing exactly which K values were more and less sensitive to changes in value, we would be able to better understand the model and better think about how we could fit it to experimental data later on.

Starting off, Farnaz has created a script in MATHEMATICA with the ordinary differential equations and used dynamics to create a graph where each K value corresponded to a

slider bar. Each slider bar could be moved to change the K value and then re visualize the graph. In the beginning stages this type of output was useful to see general trends from changing K values and the script is referenced in the appendix. After looking at the MATHEMATICA model, an order of magnitude study was used to determine sensitivity of the K values by changing each by an order of 1. Additionally, more order of magnitude studies were performed to see if changing multiple K values by an order of 1 would lead to synergistic or additive effects on the graph shapes. These additive sensitivity analyses were used with one variable that had shown a change in graph shape in addition to the other K values for an iterative approach.

3.4 Results

The first sensitivity analysis changed each K value by an order of 1 compared to the control. Values for the control were established through joint efforts with Farnaz to approximate biological values for certain values related to T cell and B cell growth and decay, and others were approximated through looking at the MATHEMATICA model with the sliders.

A

```
clc
clear

timespan= [0 100];
IC= [1 1 0];
% IC in  order of T-cell CD19 T-CD19 complex

coeff1= [0.7 0.001 0.001 0.03 0.07 0.03 0.001];
% coefficients in order of Kon Koff Kd1 Kb Kk KD (divide) kd2

coeff2= [0.07 0.001 0.001 0.03 0.07 0.03 0.001];
% coefficients in order of Kon Koff Kd1 Kb Kk KD (divide) kd2


coeff3= [0.7 0.01 0.001 0.03 0.07 0.03 0.001];
% coefficients in order of Kon Koff Kd1 Kb Kk KD (divide) kd2


coeff4= [0.7 0.001 0.01 0.03 0.07 0.03 0.001];
% coefficients in order of Kon Koff Kd1 Kb Kk KD (divide) kd2


coeff5= [0.7 0.001 0.001 0.003 0.07 0.03 0.001];
% coefficients in order of Kon Koff Kd1 Kb Kk KD (divide) kd2


coeff6= [0.7 0.001 0.001 0.03 0.7 0.03 0.001];
% coefficients in order of Kon Koff Kd1 Kb Kk KD (divide) kd2


coeff7= [0.7 0.001 0.001 0.03 0.07 0.03 0.01];
% coefficients in order of Kon Koff Kd1 Kb Kk KD (divide) kd2


options=[];

% Set up for each condition keeping K division constant

[t,y1]= ode45(@odefun, timespan, IC, options, coeff1); % Control


[t2,y2]= ode45(@odefun, timespan, IC, options, coeff2); % Change Kon


[t3,y3]= ode45(@odefun, timespan, IC, options, coeff3); % Change Koff


[t4,y4]= ode45(@odefun, timespan, IC, options, coeff4); % Change K death 1


[t5,y5]= ode45(@odefun, timespan, IC, options, coeff5); % Change K birth


[t6,y6]= ode45(@odefun, timespan, IC, options, coeff6); % Change K kill


[t7,y7]= ode45(@odefun, timespan, IC, options, coeff7); % Change death 2
```

B

```
subplot(6,2,1)
plot(t,y1(:,1), "b", t,y1(:,2), "r", t,y1(:,3), "g")
title("Control Concentration v time")
xlabel("Time (hour)")
ylabel("Concentration")

subplot(6,2,2)
plot(t2,y2(:,1), "b", t2,y2(:,2), "r", t2,y2(:,3), "g")
title("Concentration v time-- Kon")
xlabel("Time (hour)")
ylabel("Concentration")

subplot(6,2,3)
plot(t,y1(:,1), "b", t,y1(:,2), "r", t,y1(:,3), "g")
title("Control Concentration v time")
xlabel("Time (hour)")
ylabel("Concentration")

subplot(6,2,4)
plot(t3,y3(:,1), "b", t3,y3(:,2), "r", t3,y3(:,3), "g")
title("Concentration v time-- Koff")
xlabel("Time (hour)")
ylabel("Concentration")

subplot(6,2,5)
plot(t,y1(:,1), "b", t,y1(:,2), "r", t,y1(:,3), "g")
title("Control Concentration v time")
xlabel("Time (hour)")
ylabel("Concentration")

subplot(6,2,6)
plot(t4,y4(:,1), "b", t4,y4(:,2), "r", t4,y4(:,3), "g")
title("Concentration v time-- Kd1")
xlabel("Time (hour)")
ylabel("Concentration")

subplot(6,2,7)
plot(t,y1(:,1), "b", t,y1(:,2), "r", t,y1(:,3), "g")
title("Control Concentration v time")
xlabel("Time (hour)")
ylabel("Concentration")

subplot(6,2,8)
plot(t5,y5(:,1), "b", t5,y5(:,2), "r", t5,y5(:,3), "g")
title("Concentration v time-- Kb")
xlabel("Time (hour)")
ylabel("Concentration")

subplot(6,2,9)
plot(t,y1(:,1), "b", t,y1(:,2), "r", t,y1(:,3), "g")
title("Control Concentration v time")
xlabel("Time (hour)")
ylabel("Concentration")

subplot(6,2,10)
plot(t6,y6(:,1), "b", t6,y6(:,2), "r", t6,y6(:,3), "g")
title("Concentration v time-- Kk")
xlabel("Time (hour)")
ylabel("Concentration")
subplot(6,2,11)
plot(t,y1(:,1), "b", t,y1(:,2), "r", t,y1(:,3), "g")
title("Control Concentration v time")
xlabel("Time (hour)")
ylabel("Concentration")

subplot(6,2,12)
plot(t7,y7(:,1), "b", t7,y7(:,2), "r", t7,y7(:,3), "g")
title("Concentration v time-- Kd2")
xlabel("Time (hour)")
ylabel("Concentration")
```

C

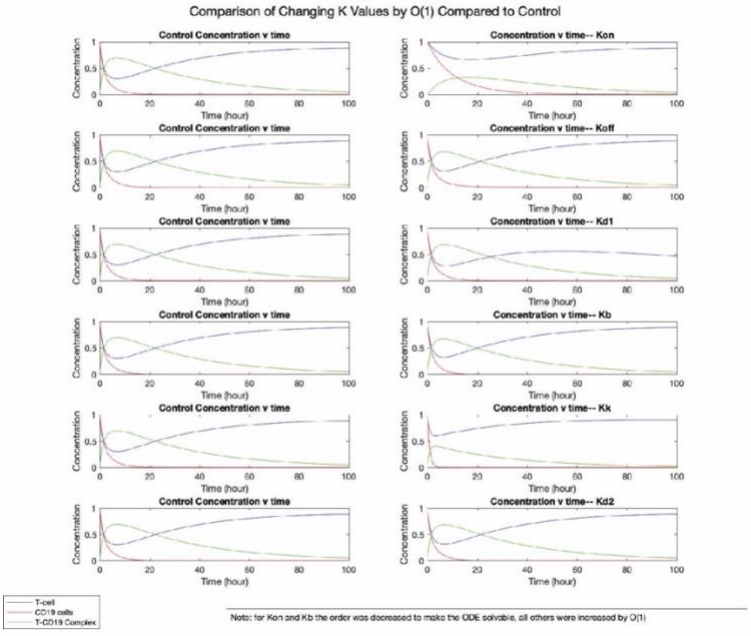


Figure 8: Order of magnitude study.

Through changing the K values by an order of 1, graphical output showed any differences or similarities between the T cell, B cell, and complex curves labeled as T-cell, CD-19 cells, and T-CD19 Complex respectively.

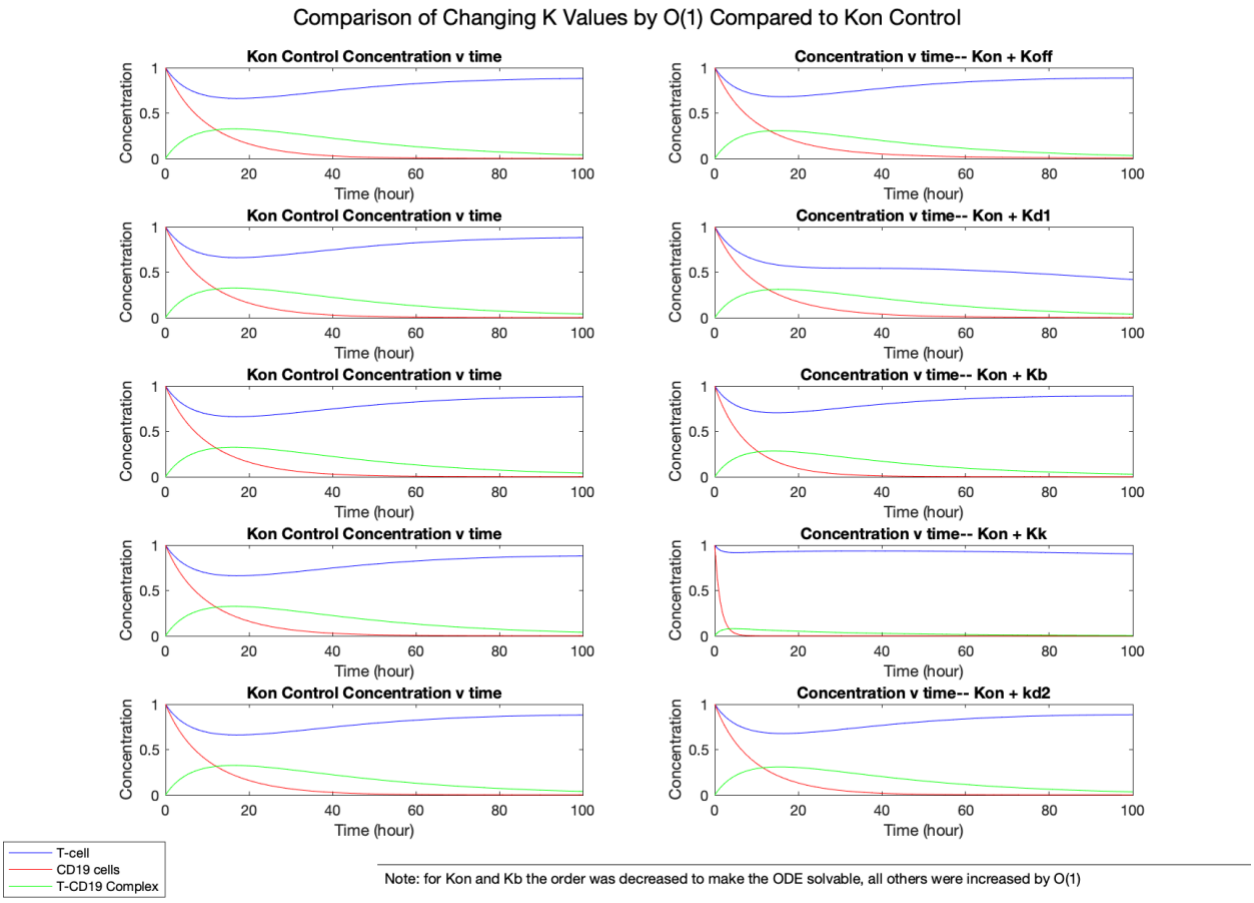


Figure 9: Order of magnitude study for synergistic affects with Kon

Through changing the K values by an order of 1 in addition to Kon being changed by and order of 1, graphical output showed any differences or similarities between the T cell, B cell, and complex curves labeled as T-cell, CD-19 cells, and T-CD19 Complex respectively.

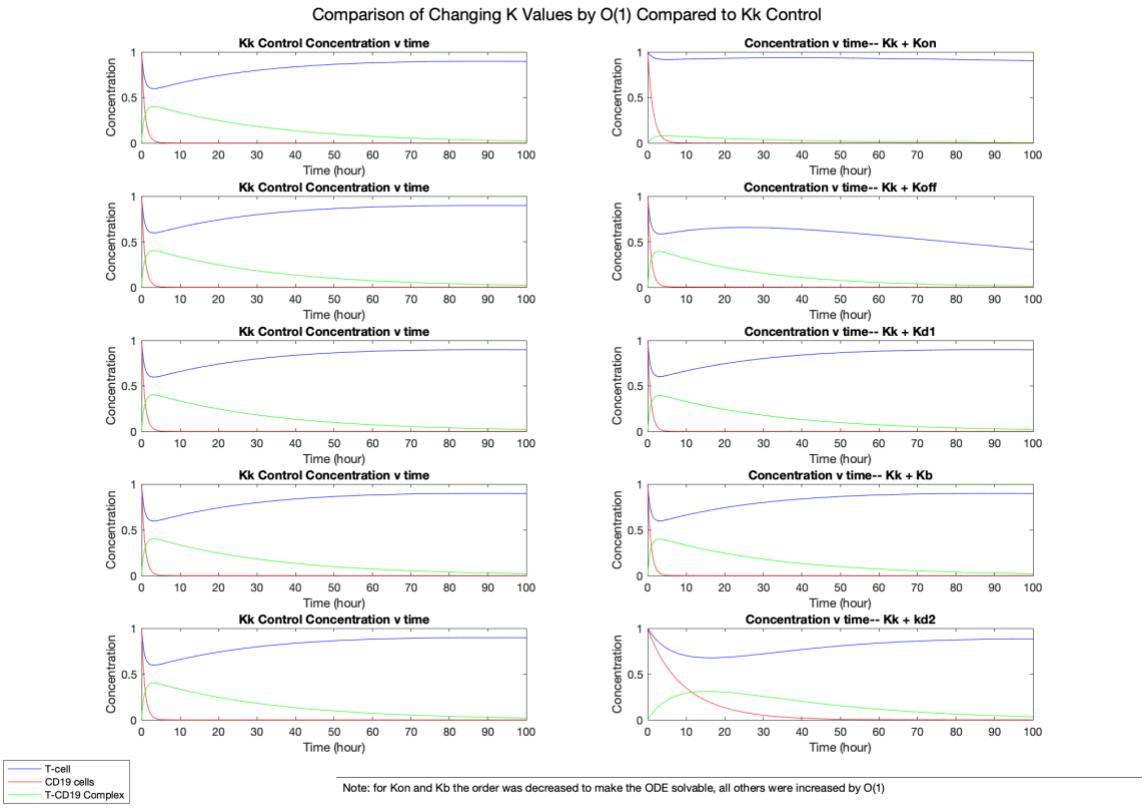


Figure 10: Order of magnitude study for synergistic affects with Kk.

Through changing the K values by an order of 1 in addition to changing Kk by an order of 1, graphical output showed any differences or similarities between the T cell, B cell, and complex curves labeled as T-cell, CD-19 cells, and T-CD19 Complex respectively.

3.5 Discussion

From the order of magnitude sensitivity studies, it appeared that the most sensitive K values on their own were Kon and Kk, as there was a visual change in the growth curves visualized. When Kon and Kk were changes by an order of 1 in addition to another variable, there were visual differences with the Kon and Kk combination as well as the Kk and Kd2

combination, showing how changing multiple K values simultaneously could change the curve shapes.

Through the creation of a model to simulate the mechanism of action of biTEs, it is possible that *in vitro* data could be fitted, and K values could be estimated to come up with an accurate representation of what happens with biTE intervention in ALL. Additionally, this model could be fitted to data from combination therapy to see how various rates change with the addition of TKIs into the system, like what is shown later in this thesis. From the model alone, it can be interpreted that the Kon or on rate of T cell B cell complex formation the Kk kill rate of B cells and Kd proliferation rate of T cells are the reason different TKIs lead to changes in the dynamics of T cells and B cells post combination therapy treatment.

Another way to interpret the results of the order of magnitude study could be to analyze the quantitative changes from graph to graph. Using a quantitative approach could add a robust way to compare the differences between conditions and add a statistically meaningful interpretation in addition to the visual one that can be seen through the graphs.

Chapter 4

The Effect of Category of TKI on Efficacy and Model

4.1 Background and Motivation

Previous research showed the antagonistic effects caused by Src TKIs in combination with biTE and synergistic effects caused by non Src TKIs in combination with biTE [9]. From experiments run by the lab and ones included in this thesis, it was hypothesized that TKIs involved with inhibiting the Src family kinases had antagonistic effects on T cells in comparison to TKIs involved with inhibiting other classes of kinases due to off target effects. One potential off target effect of the Src TKIs could result in the inhibition or decrease of T cell growth and proliferation. In order to address a decreased growth of T cells some studies have looked into supplementing with interleukins. One previous study looked into supplementing CAR-T cell therapy with IL2, IL7 and IL15 to increase CD4+ T cell activation [21]. Through this study, researchers found that the introduction of interleukins regulated genes in TCR, JAK/STAT, MAPK, AKT, and PI3K-AKT signaling, all of which play roles in cell growth and proliferation.

For these reasons it was of interest to study how combination therapy of different TKIs with biTE compared to biTE treatment and no treatment conditions, as well as explore the effects of interleukin supplementation.

4.2 Src TKI Potential to Inhibit Pathway Through pSTAT5

As mentioned, TKIs act to inhibit the phosphorylation of tyrosine kinases and are involved with inhibiting various pathways such as JAK/STAT, also known as Janus Kinase/ Signal Transducer and Activation of Transcription [12]. Previous work suggested that STATs can be activated by growth factor receptors as well as members of the Src family kinases such as c-Src [22]. Work has also shown that STAT3 and STAT5 play a role in acute and chronic leukemia, and that STAT5 is a target for some Src specific TKIs [23]. Because STAT5 activation promotes CD4+ and CD8+ T cell growth and proliferation, it is possible for TKIs involved with inhibiting phosphorylation of STAT5 to also decrease rates of T cell growth [24].

4.3 Cell Count Assay and Model Fitting Background

A cell count combination treatment assay was carried out to collect data on the growth or decay of T cells and B cells over time when treated with biTE and combination therapy. The objective of these experiments was to collect data that could be fitted to the model created in chapter 3 and if the model was a good fit for the data. The cell count assay, described in the methods section comprised of 4 conditions, no treatment, biTE treatment, combination treatment with a non Src TKI, and treatment with a Src TKI. These conditions were designed through using co-cultured PBMCs and BV173 cells stimulated with dynabeads udder no treatment, treatment with blinatumomab, blinatumomab plus nilotinib and blinatumomab plus dasatinib. Nilotinib is a TKI that is not play a role in inhibiting Src family kinases, while dasatinib does. Nilotinib is a second generation TKI designed to inhibit the BCR-ABL protein, a protein believed to cause

Ph+ ALL [25]. Dasatinib is a Src inhibitor that has been widely studied and is approved for the treatment of PH+ ALL and also inhibits BCR-ABL [26].

With the data obtained from the experiments, curve fitting of the model generated in chapter 3 was performed. This curve fitting was particularly of interest to see how the dynamics of T cells and B cells would change or stay the same across treatment conditions and see if we could fit our theoretical model to the experimental results. A first approach to fitting the data was using Maximum Likelihood Estimation (MLE) with a Poisson distribution. This was potentially of interest because the data collected from *in vitro* experiments looked like they could follow a Poisson distribution. Additionally, some researchers have studied cell proliferation dynamics and fitted Poisson distribution models to the results, which led to our potential interest in doing something similar. Full results of my coding efforts are in the appendix.

Ultimately, it was of interest to fit the initially developed model to the data. Through adding in a logistic growth term to the initial set of differential equations, a model could be fit. Through using MLE values were estimated for the K values in the model, and the division rate of T cells with the Src TKI treatment and BiTE treatment were compared.

4.4 Cell Count Assay and Model Fitting Results

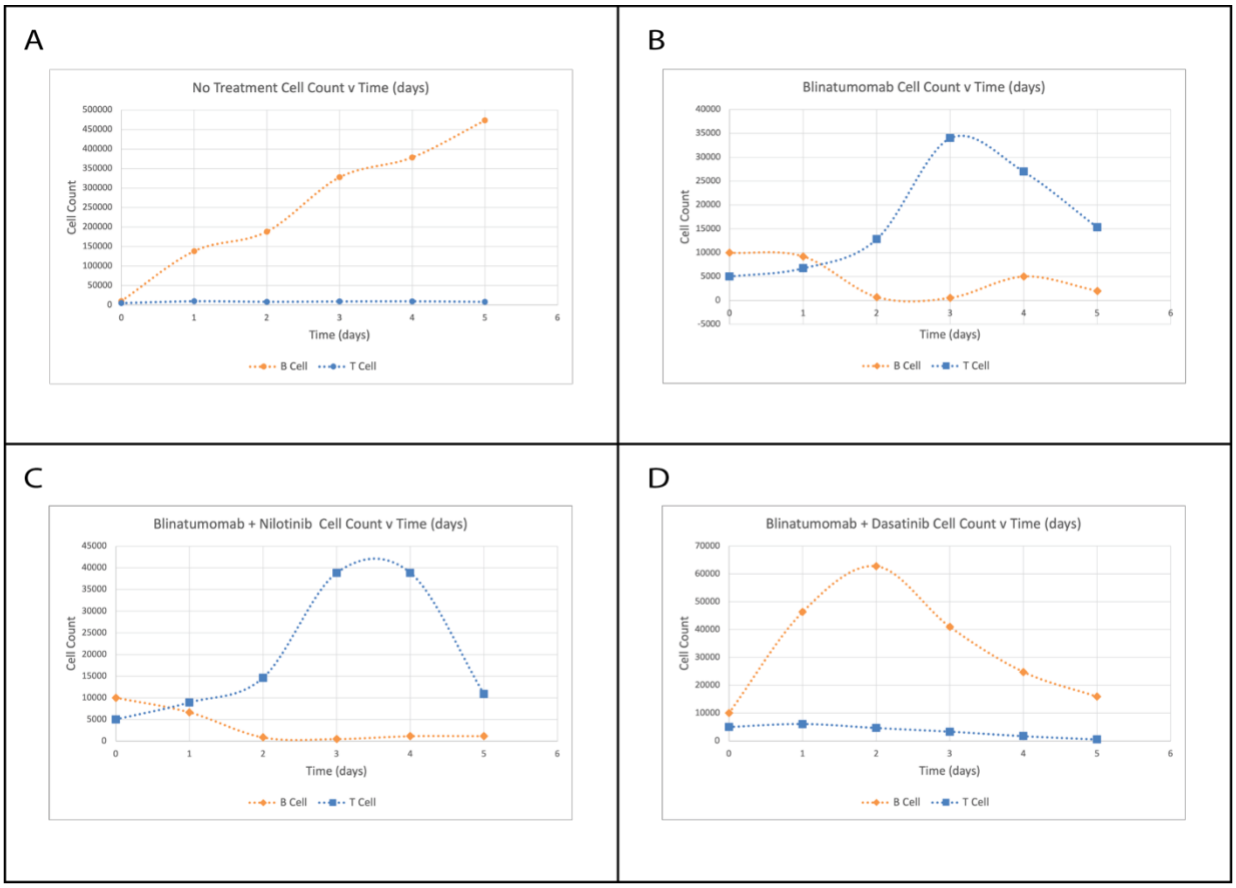


Figure 11: Combination treatment effect on growth of T cells and B cells.

Figure 11 shows the effects of combination therapy on PBMC derived T cells and BV173 B cells that were cocultured together as described in the methods section. (A) T cells (blue) and B cells (orange) with no treatment were measured over 5 days. (B) The effect of therapy with blinatumomab on T cells (blue) and B cells (orange) was measured over 5 days. (C) The effect of therapy with blinatumomab and nilotinib on T cells (blue) and B cells (orange) was measured over 5 days. (D) The effect of therapy with blinatumomab and dasatinib on T cells (blue) and B cells (orange) was measured over 5 days.

Through therapy on cocultured B cells and PBMC derived T cells, the effects of no treatment, BiTE treatment (blinatumomab), and combination treatment with a Src TKI (dasatinib) and non Src TKI (nilotinib) were measured. Through no treatment, there was a relatively constant number of T cells over 5 days and a constant increase in B cells. With BiTE

treatment, there was an overall increase in T cells and decrease in B cells over 5 days. The combination treatment with the non Src TKI nilotinib, there was an overall increase in T cells and decrease in B cells like the BiTE treatment with a slightly increased growth and decay of T cells and B cells respectively. The combination therapy with the Src TKI dasatinib led to a decrease in T cells and an increase in B cells over the 5-day period.

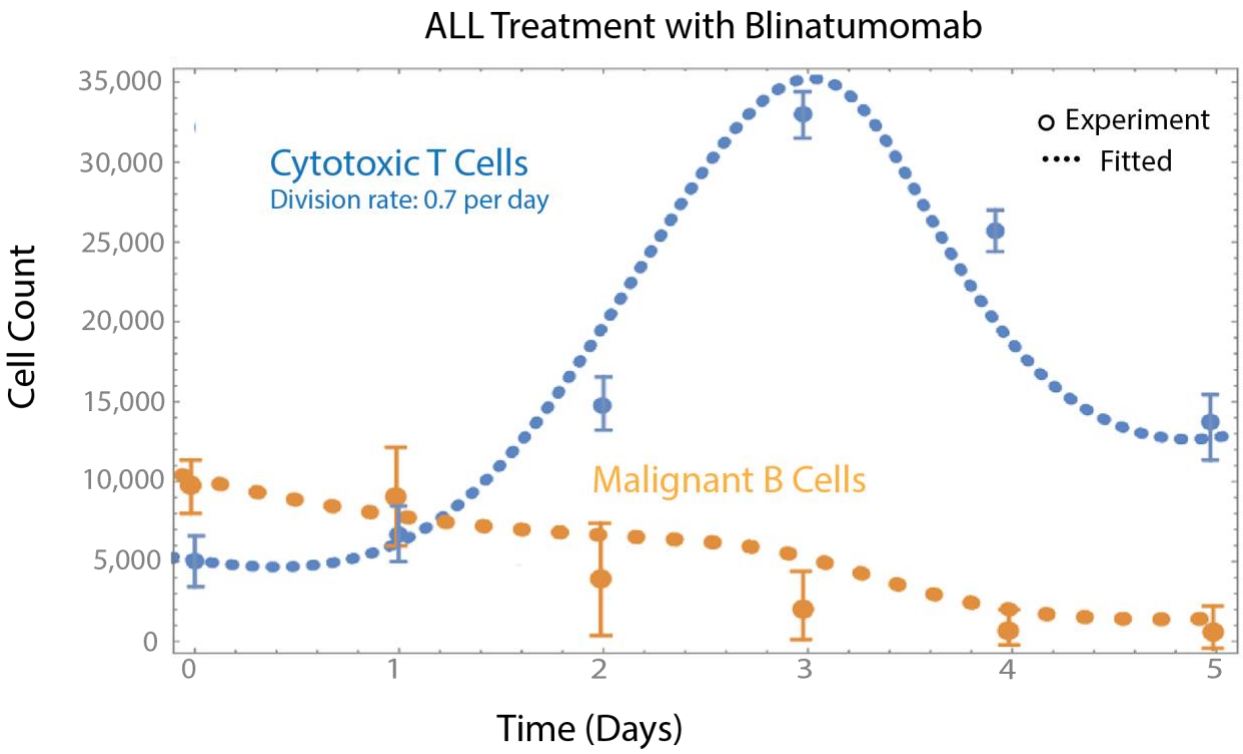


Figure 12: ALL treatment curve fitting with blicocyto.

The data obtained from the cell count assay was curve fitted using MLE with a Gaussian fit in Matlab as described above and the Kd division rate of T cells was determined to be close to 0.7 per day for the BiTE blinatumomab treatment.

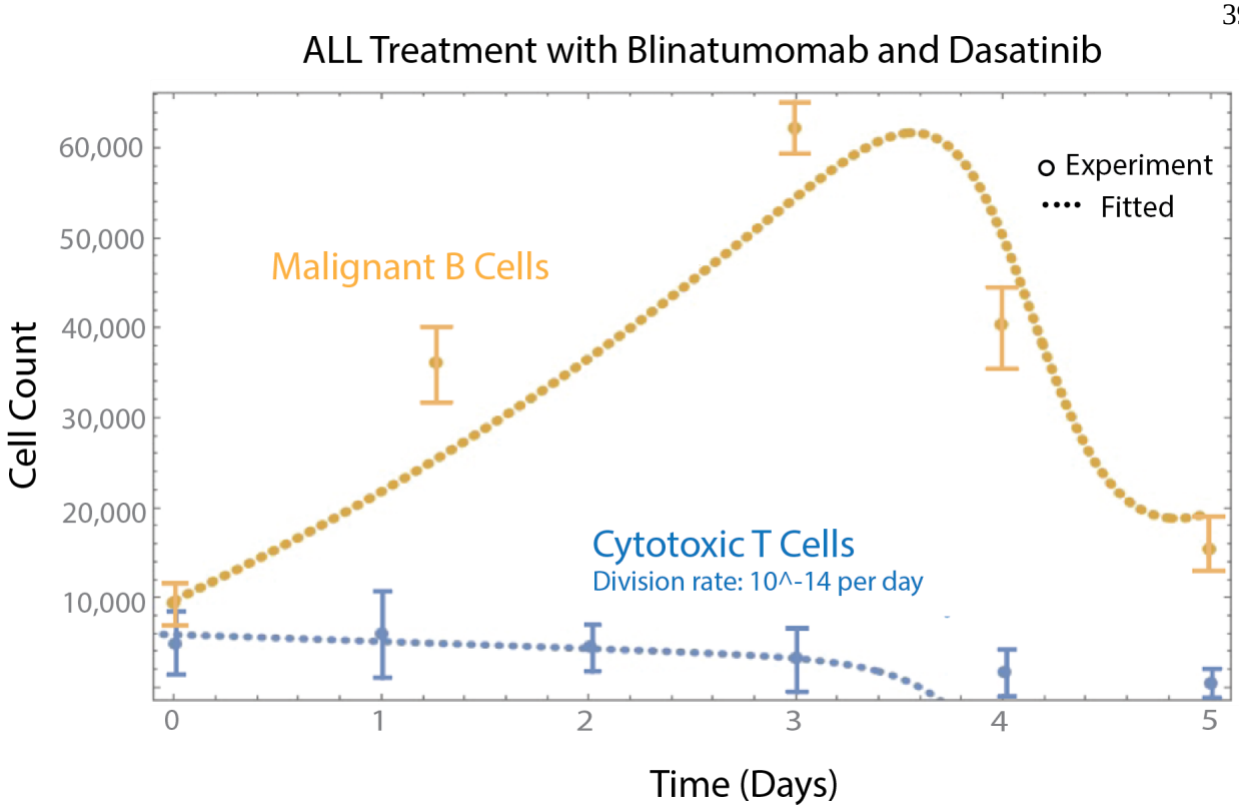


Figure 13: ALL treatment curve fitting with blicocyto and dasatinib therapy.

The data obtained from the cell count assay was curve fitted using MLE with a Gaussian fit in Matlab as described in the section above and the Kd division rate of T cells was determined to be close to 10^-14 per day for the BiTE TKI combination therapy with blinatumomab and dasatinib.

Through curve fitting the cell count data, it was found that the Kd division rate of T cells with the BiTE treatment of blinatumomab was 0.7 per day compared to the BiTE combination therapy with blinatumomab and dasatinib, where the Kd division rate of T cells was 10^-14 per day. This decrease in estimated Kd by curve fitting further suggests the antagonistic effects of Src TKIs on combination therapy with a BiTE.

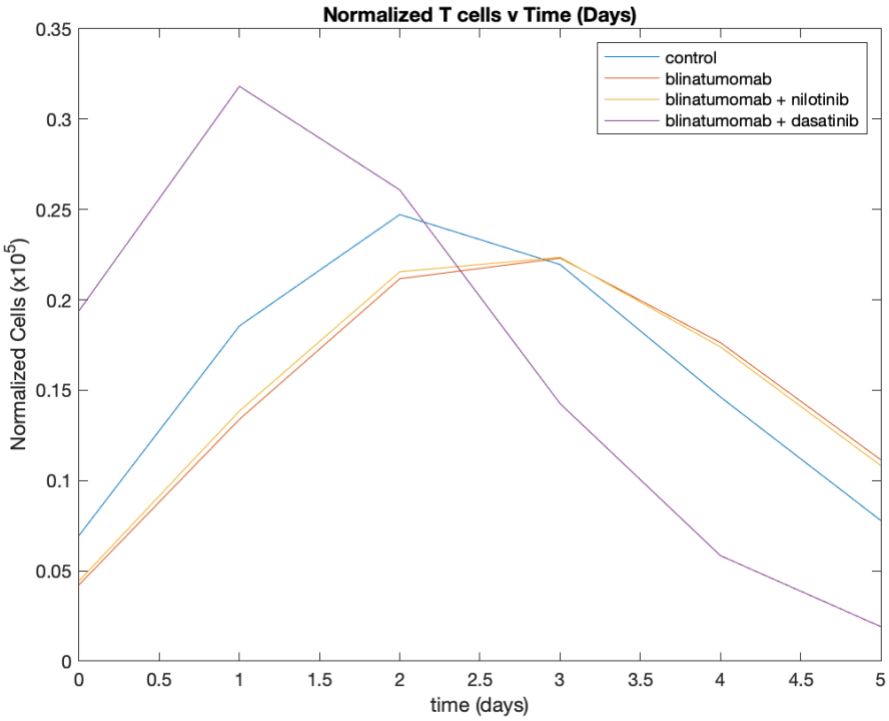


Figure 14: Curve fitting of T cell data with Poisson distributed MLE in Matlab.

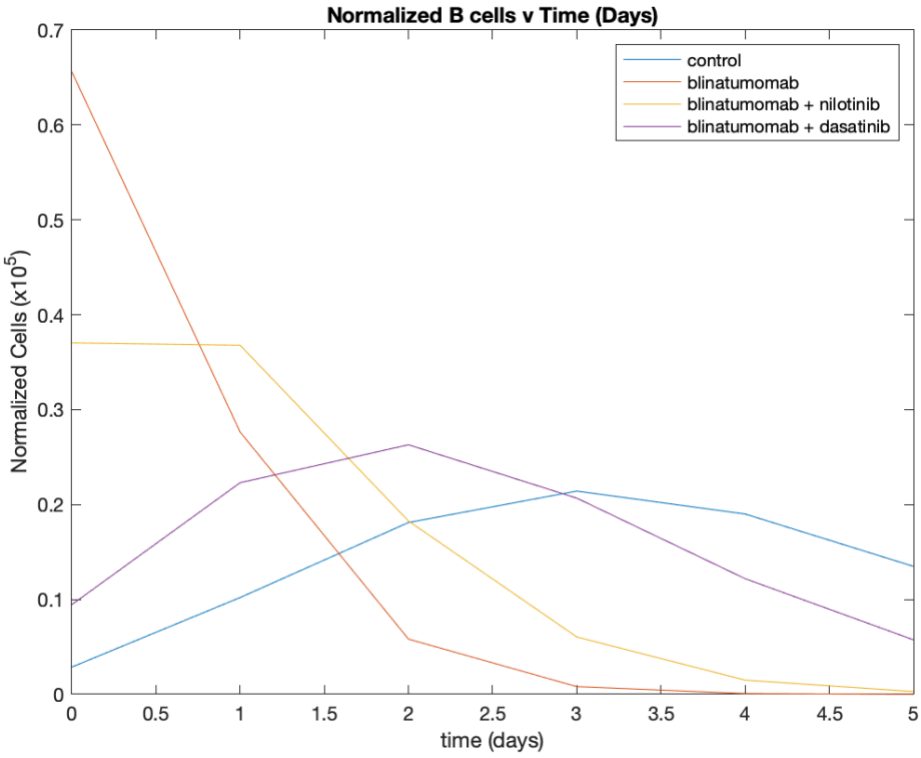


Figure 15: Curve fitting of B cell data with Poisson distributed MLE in Matlab.

Through using Maximum Likelihood Estimation in Matlab with a Poisson distribution, the fitting parameter of lambda, also known as the average number of events that occur over an interval. When lambda is small, there is a steep incline or decline in a curve, when it is large, there is a shallower shape to the curve [27]. From fitting the experimental data, the control T cell had an estimated lambda value of 2.7 and a B cell lambda value of 3.33. The blinatumomab T cell had an estimated lambda value of 3.2 and a B cell lambda value of 0.42. The blinatumomab plus nilotinib T cell had an estimated lambda value of 3.1 and a B cell lambda value of 0.99. The blinatumomab plus dasatinib T cell had an estimated lambda value of 1.6 and a B cell lambda value of 2.3. This suggests, the rate of T cell growth in the blinatumomab plus dasatinib condition is lower than the other conditions as the lambda value is the lowest. The curve fitting also suggests the blinatumomab plus dasatinib condition has a higher growth rate compared to the blinatumomab plus nilotinib and the blinatumomab conditions, as the lambda value is higher. The graph also shows how the blinatumomab plus dasatinib condition has the greatest rate of growth for B cells compared to all conditions.

4.5 Western Blot to Test IL Rescue Background

In order to test whether Src related TKIs contribute to off target effects, a series of Western Blot experiments were carried out on Jurkat cells to see if phosphorylation of LCK was impacted by the category of TKI. In some experiments, a series of interleukins (IL) were added

to attempt to rescue any off-target effects caused by the TKIs. Candidates for the IL rescue experiments included IL2, IL7, IL15, and a combination of the three called ILC.

IL2 was studied because of its role in stimulating cell proliferation of immune cells including T cells. IL2 is typically produced by activated T cells, dendritic cells and B cells [28]. IL7 was studied because of its role in stimulating cell proliferation of T cells in addition to other immune cells. IL7 was also of interest to study because immune cells do not typically produce a lot of it [29]. IL15 was studied because of its role in stimulating T cell proliferation. IL15 is known to be produced by dendritic cells, monocytes and epithelial cells and plays a role in maintaining homeostasis of the immune system [30]. A combination of the three was also studied because the three are involved in activating pathways that promote the survival and proliferation of immune cells. One of the major pathways involved with this is the JAK/STAT pathway, which was studied through staining for pSTAT5 in the experiments below [23].

Optimization assays for best time dosing of interleukin in addition to TKIs were performed by Farnaz Naeemikia. Farnaz played a crucial role in data collection for the Western Blots displayed below in figures 10-13 and helped me run the IL2 rescue assay. More Western Blots were run by me independently, but due to storage issues and nonspecific binding, they are not in the results section. One replicate of a 2 hour treatment IL2 rescue has been moved to the appendix section rather than the results to show my independent experiment. The Western Blots were analyzed in FIJI by me and Mikayla and we created the bar graphs below. All figures were designed independently although the data Mikayla and I represent is the same.

To quantify normalized ratios, the intensity of each sample was quantified using FIJI and normalized to the loading controls, then the values generated were divided by the value for the negative control.

4.6 Western Blot to Test IL Rescue Results

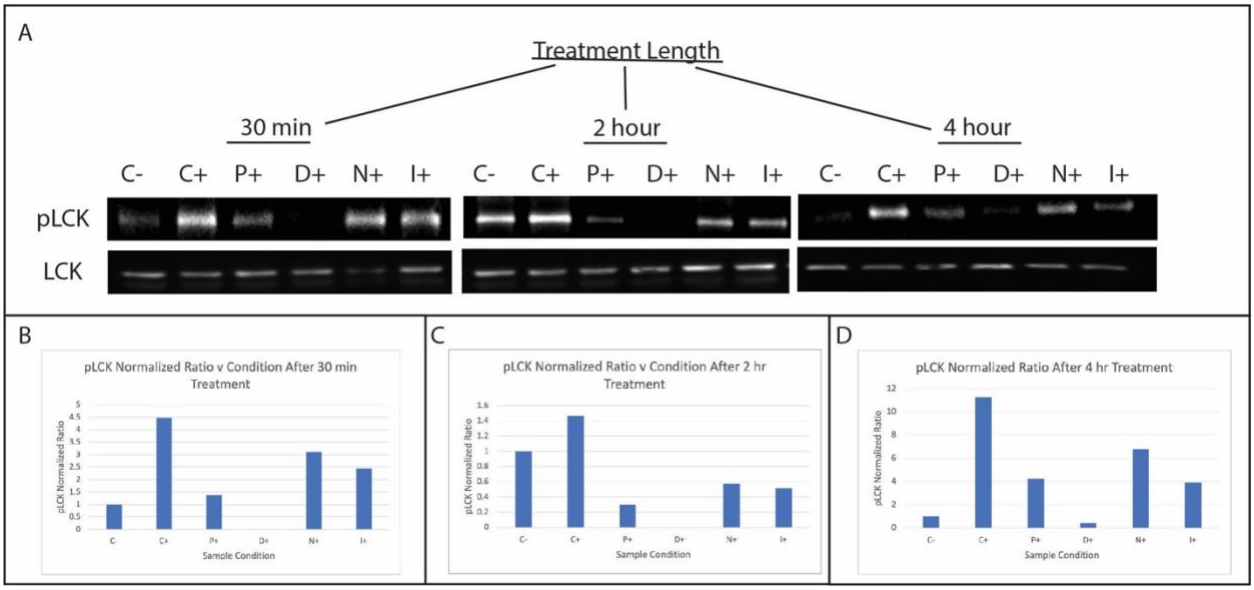


Figure 16: Treatment length optimization assay.

(A) Three western blots were performed with Jurkat cells treated with Ponatinib, Dasatinib, Nilotinib and Imatinib either for 30 minutes, 2 hours or 4 hours. (B-D) Western blot data was analyzed and normalized to the loading control as well as the negative control and plotted as pLCK normalized ratio versus sample condition.

In the optimization assay shown in Figure 10, the greatest visual difference between blots was seen in the hour treatment. Graphs in B-D support this visual difference between the blots and show the most differences between the conditions. There was a potential transfer issue in the 30 minute blot from the gel to membrane. The blot data suggests an overall trend of Src TKI with a lower normalized pLCK ratio compared to non Src TKI and positive control.

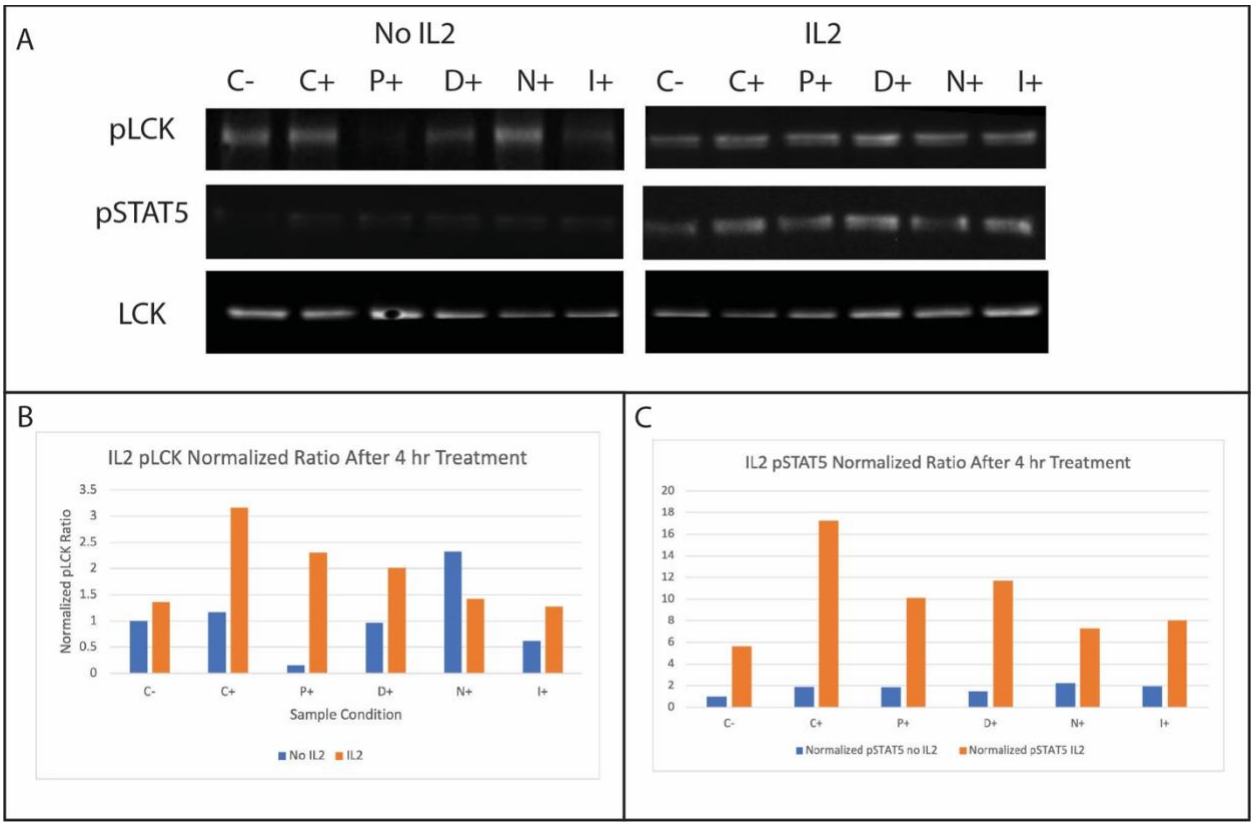


Figure 17: IL2 rescue experiment.

In the IL2 rescue experiment, pLCK and pSTAT5 were measured against a loading control of LCK. Without IL2, there was a lower normalized pLCK ratio for Src TKIs compared to the positive control and Nilotinib, but not Imatinib. With IL2, there was also an increase in pLCK for all conditions other than nilotinib. Without IL2, there were similar normalized pSTAT5 ratios, with a slightly lower ratio for ponatinib and dasatinib compared to nilotinib and imatinib. With IL2, there was an increased pSTAT5 ratio across all conditions.

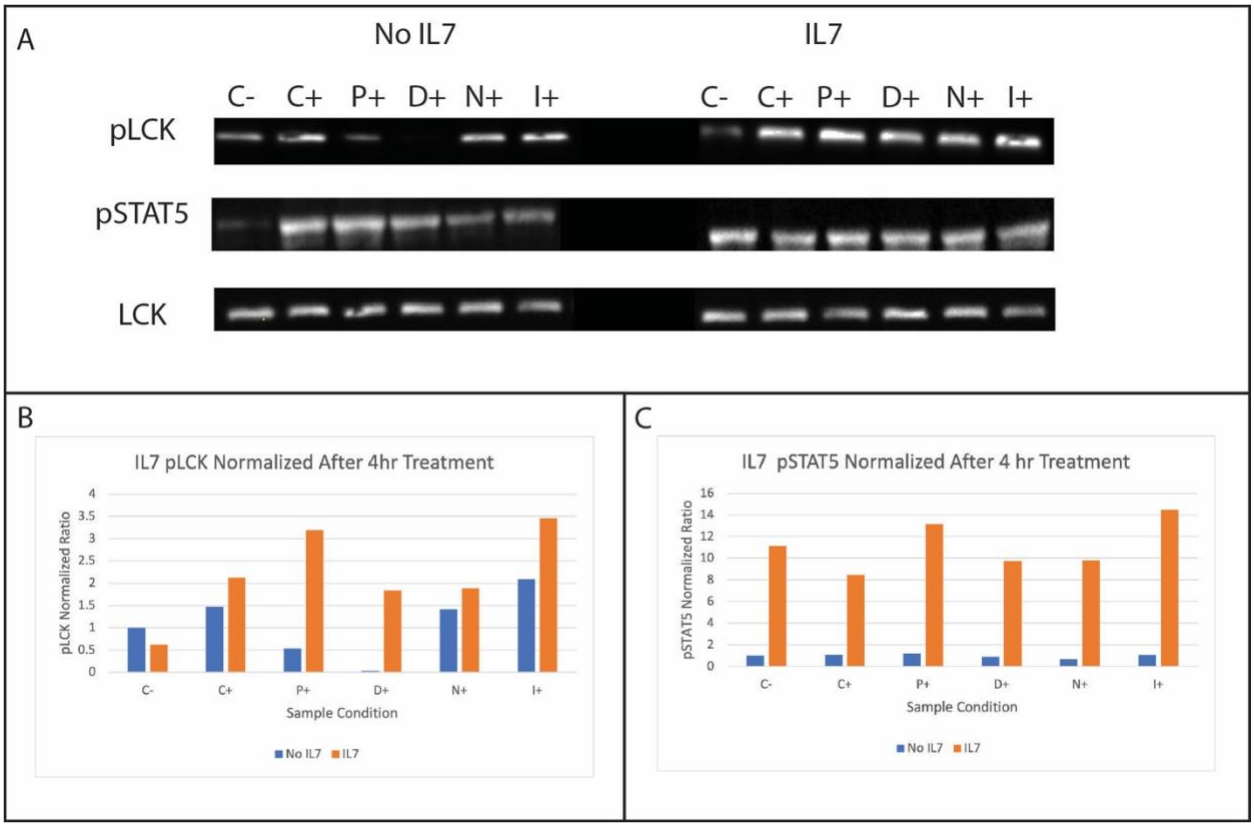


Figure 18: IL7 rescue experiment.

In the IL7 rescue experiment, pLCK and pSTAT5 were measured against a loading control of LCK. Without IL7, here was a lower normalized pLCK ratio for the Src TKIs compared to positive control and compared to nilotinib and imatinib. With the addition of IL7, there were increased levels of pLCK for all conditions other than negative control. Without IL7, there were similar normalized pSTAT5 ratios across all conditions. With the addition of L7, there were increased normalized pSTAT5 ratios across all conditions.

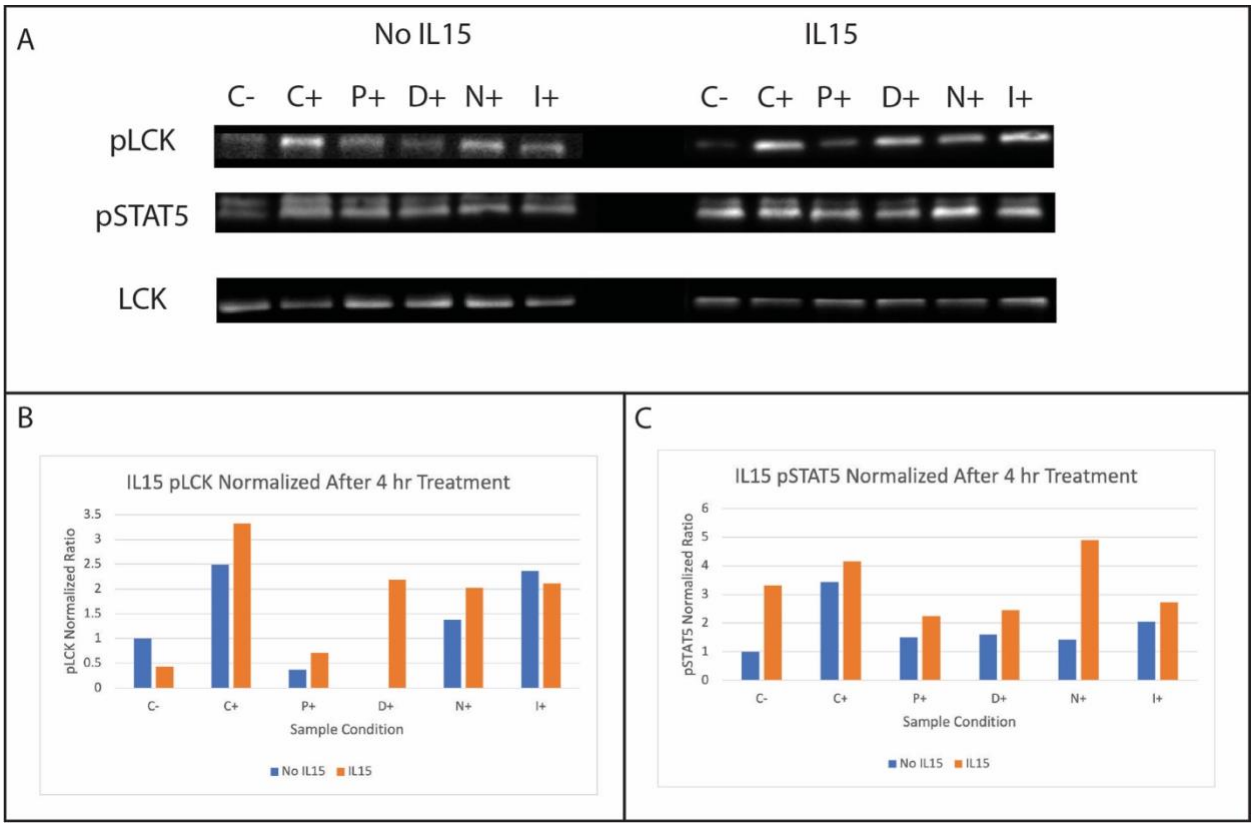


Figure 19: IL15 rescue experiment.

In the IL15 rescue experiment, pLCK and pSTAT5 were measured against a loading control of LCK. Without IL15, there was a lower normalized pLCK ratio for the Src TKIs compared to the positive control and nilotinib and imatinib conditions. With the addition of IL15, there was an increased measurement of pLCK for all conditions other than the negative control. Without IL15, the normalized pSTAT5 ratio was slightly lower for ponatinib and dasatinib and nilotinib compared to positive control and imatinib. With the addition of IL15, there was an increased normalized pSTAT5 ratio across all conditions.

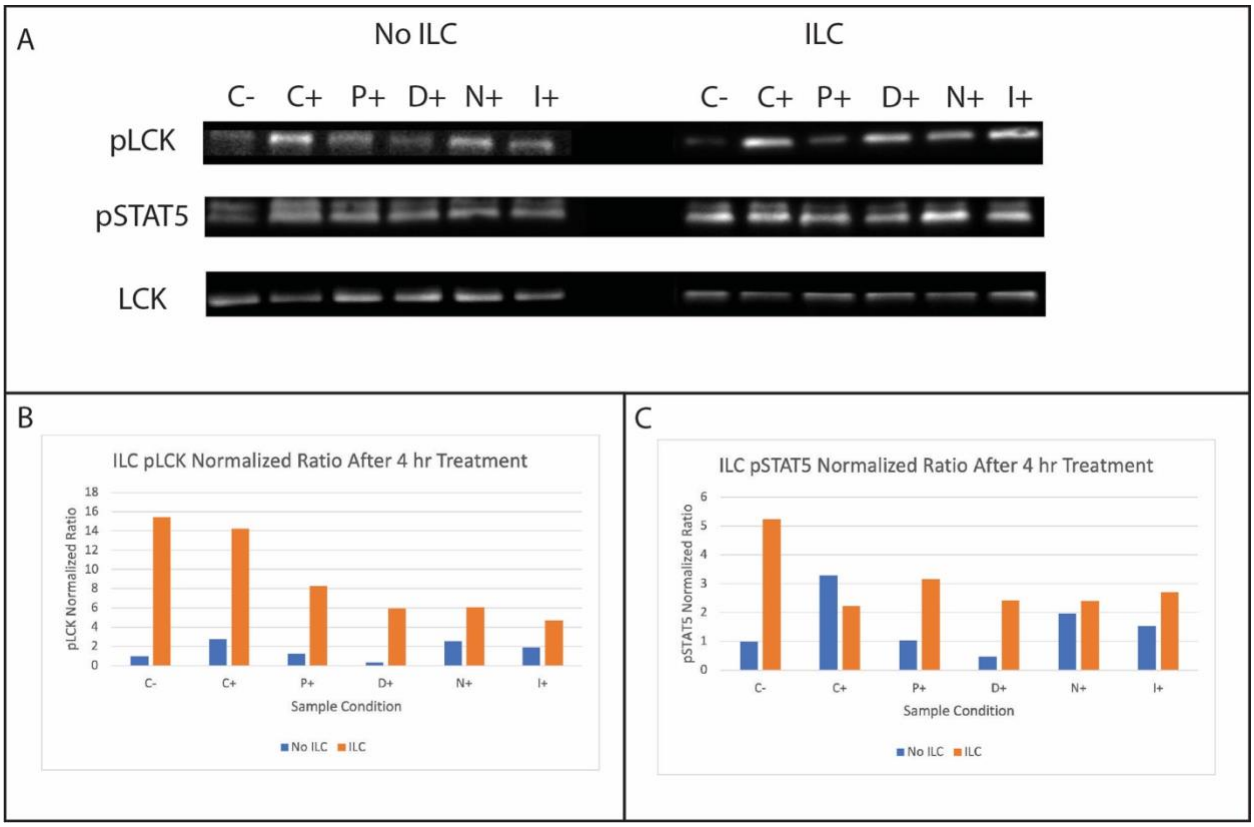


Figure 20: ILC rescue experiment.

In the ILC rescue experiment, pLCK and pSTAT5 were measured against a loading control of LCK. Without ILC, there was a lower normalized pLCK ratio for the Src TKIs compared to positive control and nilotinib and imatinib. With the addition of ILC, there was an increase in normalized ratio of pLCK for all conditions. Without ILC, the normalized pSTAT5 ratio was lower for ponatinib and dasatinib compared to nilotinib and imatinib and positive control. With ILC, there was an increased normalized pSTAT5 ratio across all conditions except for the positive control.

4.7 Discussion

Through the cell count assay with combination therapy, there are visual differences between the Src TKI combination therapy and the non Src combination therapy and biTE only treatment. In the biTE only treatment and the biTE plus nilotinib treatment, there were increases in the T cell growth over the course of 5 days compared to the control. This suggests that both biTE in its own and in combination with nilotinib leads to a greater amount of T cell growth and proliferation. With these two treatments, there was also a decrease in malignant B cells over time in comparison to the control. This would suggest that these treatments lead to a greater inhibition of B cell proliferation. On the other hand, the combination of biTE and dasatinib led to a decrease in T cells over time and an increase in malignant B cells over time in comparison to the control. This would suggest the combination therapy with dasatinib has antagonistic effects. The modeling data supports this as the T cell division date decreased from the biTE condition to the biTE plus dasatinib from 0.7 per day to 10^-14 per day. This further suggests how the computational model supports the results found in *in vitro* experimentation, and that the division rate of T cells is impacted negatively by the Src TKIs in comparison to the non Src TKIs. Additionally, the data suggests a decrease in B cell killing with the Src TKI in comparison to the control and non Src TKI, which agrees with the order of magnitude study, showing the Kk or killing rate of B cells as a key indicator of T cell and B cell dynamics.

The curve fitting code was designed to fit the data to the model and better understand how the T cell and B cell dynamics changed due to the type of treatment given to the cells. Through better optimizing the code, a better fit could be made to fit the parameters and simplify the model further. Further using the computational model to study how dynamics are affected by

the type of combination treatment will lead to a more mechanistic approach to potentially support the hypothesis that Src TKIs, when used in combination with biTE, cause a decrease in T cell proliferation (or cause off target effects on T cells) as well as decrease B cell killing, ultimately leading to an overall decrease in T cells and an increase in B cells.

Ultimately, the western blot data suggests that IL2, IL7, IL15, and ILC have effects on increasing pLCK and pSTAT5 activation. To show statistical significance more replicates need to be run, but examining the data we see an increase in pLCK and pSTAT5 activation with added interleukin across most experiments.

While the results appeared to agree with the idea that interleukin supplementation supports increased activation of the pSTAT5 pathway and phosphorylation of LCK, there is some room for error in the experiments. One source for potential error with the staining of western blots was potential for nonspecific binding or insufficient blocking time, as this could lead to artifacts in the final blot imaging. Additionally, one source of potential error was not leaving enough time for primary and secondary antibodies to bind, which could lead to a less bright band in the final image of the blot. Another reason some blots may have not been as bright as others could have been a transferring error, or not allowing enough time for the blot to transfer onto the membrane completely. In order to limit the amount of error, the blots could be repeated, and care could be taken to ensure these mistakes do not happen. Additionally, the western blots were performed on Jurkat cells, which are known to have different properties from PBMC derives T cells. Therefore the results of these experiments can not be directly applied to PBMC derived T cells, and the experiments should be repeated on T cells isolated from PBMCs.

Chapter 5

Conclusions and Future Directions

5.1 Summary of Findings

Through the model creation, a set of ordinary differential equations were derived and a model in MATLAB was created. Through this model, the order of magnitude study showed that the Kon value of T cell CD19 complex formation and the Kk value of CD19 cell killing. This study suggests the Kon and Kk values are most indicative of the dynamics and are sensitive parameters.

Through the multiple parameter sensitivity analysis studies, it was found that the combination of Kon value of T cell CD19 complex formation and the Kk value of CD19 cell killing as well as the Kk value of CD19 cell killing and Kd2 value of T cell proliferation. This suggests that these combinations could be synergistic in nature and the combined effects of the changes K values cause a greater change in dynamics compared to altering the individual parameters.

Through *in vitro* experiments, the cell growth of T cells and CD19 cells were observed over 5 days. Without treatment, there was a relatively constant amount of T cells and a linear appearing increase in CD19 cells. Through BiTE treatment with blinatumomab and combination treatment with blinatumomab and nilotinib, there was an increase in the number of T cells and a decrease in the amount of CD19 cells. Through combination treatment with blinatumomab and dasatinib, there was a decrease in T cells and an initial increase in CD19

cells. This suggests positive effects when BiTE is used as well as combination treatment with a nilotinib, but antagonistic effects when a Src TKI such as dasatinib is used in combination treatment.

Through curve fitting, it was apparent that the treatment with dasatinib and blinatumomab led to a decreased T cell division or proliferation rate of Kd in comparison to the blinatumomab condition. This further suggests the idea that the combination therapy with a Src TKI contributes to antagonistic effects on T cells. In summary, these curve fitting results support the hypothesis that Src TKI combination therapy with biTE leads to a decrease in T cell proliferation and an increase in B cell growth, and that both factors are reasons for the difference in effects of the Src TKI combination therapy and non Src TKI combination therapy.

Through western blot experiments, it was found that the Src TKIs have decreased pLCK and pSTAT5 activity compared to other TKIs and BiTE when no interleukin is added. It was also found that the addition of interleukins can rescue the effects of the Src TKIs when added to the treatment. This suggests the addition of interleukin could be a beneficial addition to combination therapy, especially when a Src TKI is used. This also suggested that Src TKIs have off target effects in inhibiting pLCK activation through the pSTAT5 pathway. In many of the conditions, the interleukin supplementation also increased pSTAT5 and pLCK activity across the non Src conditions, suggesting interleukin addition could be useful for multiple conditions.

Ultimately, the results support the hypothesis that the T cell deficit seen after Src TKI combination treatment is due to off target effects of the therapy with lack of phosphorylated LCK, and not due to the depletion of B cells. In fact, with the Src TKI combination

treatment, the growth rate of B cells increases, which potentially contributes to a further decrease in T cell division and leads to antagonistic effects.

5.2 Future Work

There are various routes combination therapy treatment can go in the near future and using the results of experiments in this thesis could help with exploring these new directions. One route to be explored is *in vivo* what happens to patients when interleukins are used to supplement combination therapy. It would be interesting to see if a therapy cocktail of TKI, BiTE, and interleukins would improve treatment outcomes for patients suffering from ALL. Additionally, it is of interest to see if the results found in this thesis are repeatable *in vitro* with patient cells. This would give researchers a better idea of how ALL patient cells react to this regimen of combination therapy. Because most experiments were run with Jurkat cells and BV173 cells, it would be of interest to repeat all experiments with PBMCs as well as isolated patient T cells to see if there are any differences. It is important to keep in mind that experiments performed with Jurkat cells cannot be directly compared to PMBC or isolates T cell experiments and would need to be run on PBMCs or isolated T cells to have result comparisons.

Another aspect to examine is the effect of time-dosing cells with the various drugs. It would be of interest to see how treatment results are affected by the order of treatment with TKI, BiTE, and interleukin to see if there is an optimized way to dose patients for the best outcome. The Western Blot experiments touched on optimizing the assay to 4 hours, but it could be of interest to run all experiments with 30 min, 2 hours, and 4 hours of treatment to see how each outcome changes over time. Another route with time dosing could be to add TKI, BiTE and

interleukin at different times and see the effects when the three therapeutics are added at different time points. This could help determine if there is a certain order for effective time dosing as well as seeing if there is a timepoint when the interleukin rescue is most or least effective.

In addition to these avenues, it would also be of interest to repeat the experiments by treating cells using viscometry to simulate blood flow [31]. This is of interest because the therapeutics would eventually be put into the circulation system of a patient, and it would be of interest to simulate the circulation process while drugs are being administered [32]. This could give a more accurate perspective on how a patient's system would react to the drug combinations before treating real patients with the drugs.

Appendix A

Mathematica model

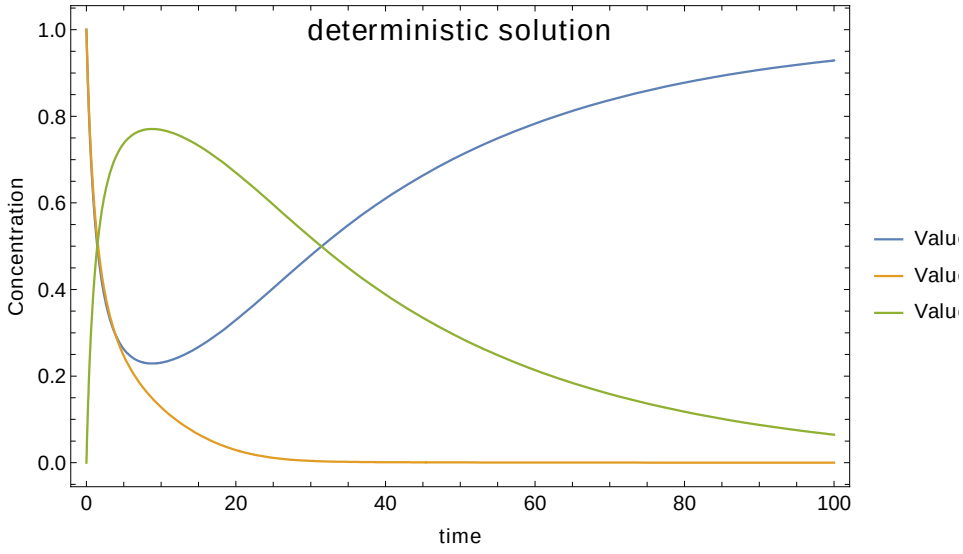
In[58]:=
```
ClearAll[TD, Tcell, complex]
```

In[59]:=
```
TcellCD19[{kd1_, kd2_, kb_, kk_, kD_, kon_, koff_, Tcell0_, CD190_, complex0_}] :=
 {Tcell'[t] ==
   kD * complex[t] - kd1 * Tcell[t] - kon * Tcell[t] * CD19[t] + koff * complex[t],
  CD19'[t] == - kon * Tcell[t] * CD19[t] + koff * complex[t] + (kb - kd2) * CD19[t],
  complex'[t] == kon * Tcell[t] * CD19[t] - koff * complex[t] - kD * complex[t],
  Tcell[0] == Tcell0, CD19[0] == CD190, complex[0] == complex0
 }
sol1 = NDSolve[TcellCD19[{0.0001, 0.0001, 0.03, 0.07, 0.03, 0.7, 0.001, 1, 1, 0}],
  {Tcell[t], CD19[t], complex[t]}, {t, 0, 100}]
```

Out[60]=
```
{{Tcell[t] → InterpolatingFunction[ 

 ][t],

  CD19[t] → InterpolatingFunction[ 

 ][t],

  complex[t] → InterpolatingFunction[ 

 ][t]}}
```

In[61]:=
```
Plot[{Values[sol1[[1]][[1]]],
  Values[sol1[[1]][[2]]], Values[sol1[[1]][[3]]]}, {t, 0, 100},
 PlotTheme → "Detailed", FrameStyle → GrayLevel[0],
 FrameTicksStyle → GrayLevel[0], FrameTicksStyle → GrayLevel[0],
 Frame → True, FrameLabel → {Style["time", Black, Bold, FontSize → 14],
   Style["Concentration", Black, Bold, FontSize → 14]},
 LabelStyle → Directive[Black, Bold, FontSize → 14], ImageSize → Large,
 Epilog → Text[Style["deterministic solution", Black, Bold, 20],
   Scaled[{.5, 0.95}]], PlotRange → All]
```

Out[61]=



In[62]:=
```
Solve[{0 → kD * complex - kd1 * Tcell - kon * Tcell * CD19 + koff * complex,
  0 → - kon * Tcell * CD19 + koff * complex + kb * CD19 - kd2 * CD19,
  0 → kon * Tcell * CD19 - koff * complex - kD * complex}, {Tcell, CD19, complex}]
```

Out[62]=
```
{{Tcell → 0, CD19 → 0, complex → 0}}
```

In[63]:=
```
f1[Tcell_, CD19_, complex_] :=
 kD * complex - kd1 * Tcell - kon * Tcell * CD19 + koff * complex
f2[Tcell_, CD19_, complex_] :=
 - kon * Tcell * CD19 + koff * complex + kb * CD19 - kd2 * CD19
f3[Tcell_, CD19_, complex_] := kon * Tcell * CD19 - koff * complex - kD * complex
```

In[66]:=
```
j =
 ( D[f1[Tcell, CD19, complex], Tcell]  D[f1[Tcell, CD19, complex], CD19]  D[f1[Tcell, CD
   D[f2[Tcell, CD19, complex], Tcell]  D[f2[Tcell, CD19, complex], CD19]  D[f2[Tcell, CD
   D[f3[Tcell, CD19, complex], Tcell]  D[f3[Tcell, CD19, complex], CD19]  D[f3[Tcell, CD
 ) // Simplify
```

Out[66]=
```
{{-kd1 - CD19 kon, -kon Tcell, kD + koff},
 {-CD19 kon, kb - kd2 - kon Tcell, koff}, {CD19 kon, kon Tcell, -kD - koff}}
```

In[67]:=
```
j // MatrixForm
```

Out[67]//MatrixForm=

$$\begin{pmatrix} -kd1 - CD19\,kon & -kon\,Tcell & kD + koff \\ -CD19\,kon & kb - kd2 - kon\,Tcell & koff \\ CD19\,kon & kon\,Tcell & -kD - koff \end{pmatrix}$$

In[68]:=
```
j1 = j /. {Tcell → 0, CD19 → 0, complex → 0}
```

Out[68]=
```
{{-kd1, 0, kD + koff}, {0, kb - kd2, koff}, {0, 0, -kD - koff}}
```

In[69]:=
```
j1 // MatrixForm
```

Out[69]//MatrixForm=

$$\begin{pmatrix} -kd1 & 0 & kD + koff \\ 0 & kb - kd2 & koff \\ 0 & 0 & -kD - koff \end{pmatrix}$$

In[70]:=
```
Reduce[-kd1 < 0, kb - kd2 < 0, -kD - koff < 0, kd2 > 0, kb > 0]
```

Reduce: Options expected (instead of kb > 0) beyond position 4 in Reduce[-kd1 < 0, kb - kd2 < 0, -kD - koff < 0, kd2 > 0, kb > 0]. An option must be a rule or a list of rules.

Out[70]=
```
Reduce[-kd1 < 0, kb - kd2 < 0, -kD - koff < 0, kd2 > 0, kb > 0]
```

In[71]:=

$$m = \begin{pmatrix} -kd1 - kon & -kon & kD + koff \\ -kon & -kon + kb - kd2 & koff \\ kon & kon & -koff - kD \end{pmatrix};$$

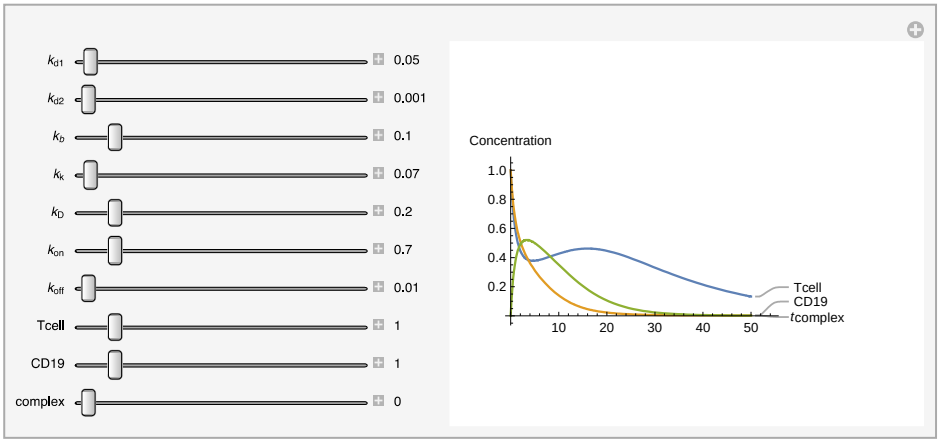
In[72]:=
```
Eigenvectors[m] // MatrixForm
```

In[73]:=
```
a = Eigenvectors[m] // Transpose;
```

In[74]:=
```
ainv = Inverse[a] // FullSimplify;
```

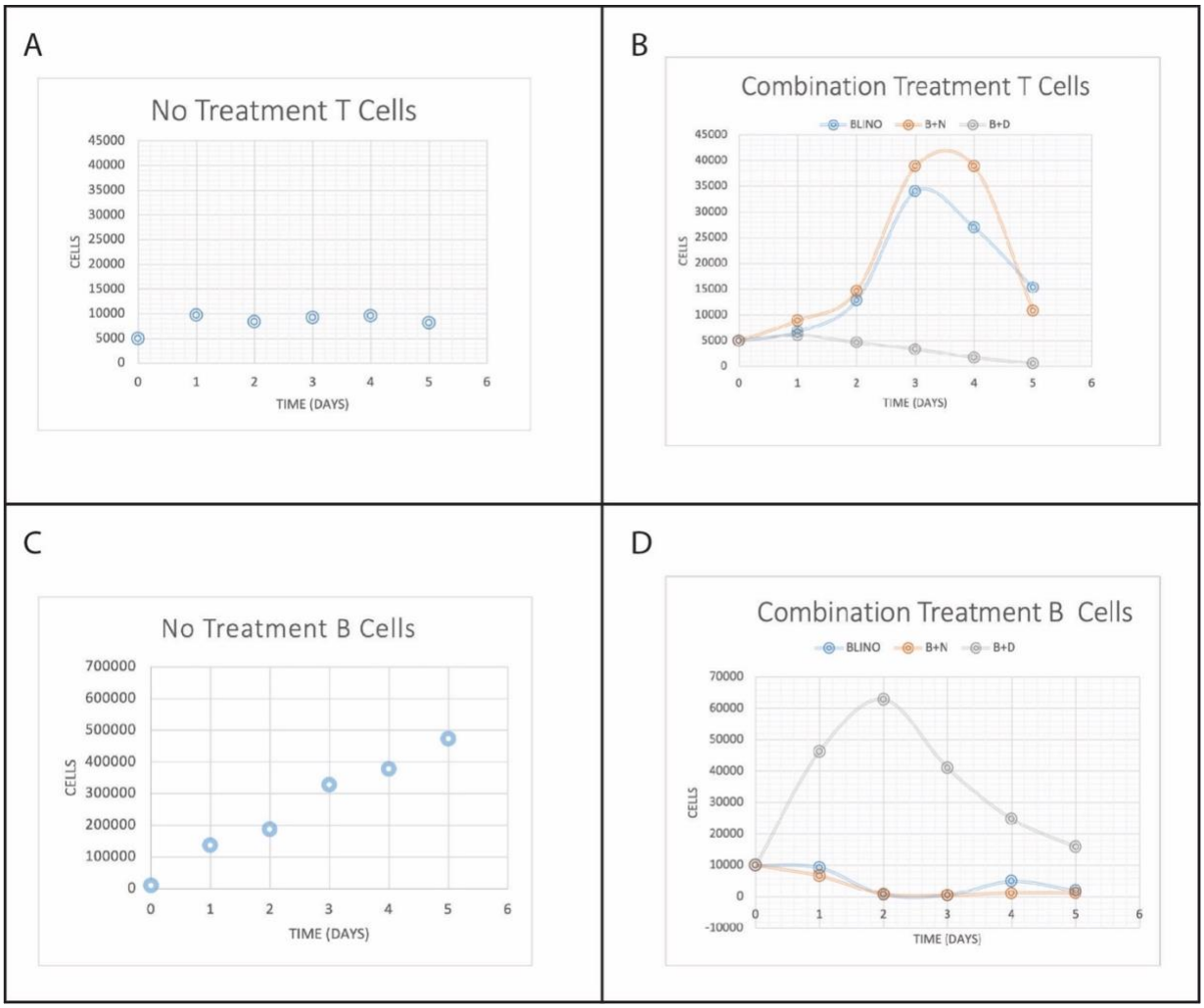
In[75]:=
```
Manipulate[sol3 =
  NDSolve[TcellCD19[{kd1, kd2, kb, kk, kD, kon, koff, Tcell0, CD190, complex0}],
   {Tcell[t], CD19[t], complex[t]}, {t, 0, 50}];
 Plot[{Values[sol3[[1]][[1]]], Values[sol3[[1]][[2]]], Values[sol3[[1]][[3]]]},
  {t, 0, 50}, AxesLabel → {t, "Concentration"},
  PlotLabels → {"Tcell", "CD19", "complex"}, PlotRange → All],
{{kd1, 0.05, "$k_{d1}$"}, 0.005, 5, Appearance → "Labeled"},
{{kd2, 0.001, "$k_{d2}$"}, 0.0001, 1, Appearance → "Labeled"},
{{kb, 0.1, "$k_b$"}, 0.001, 1, Appearance → "Labeled"},
{{kk, 0.07, "$k_k$"}, 0.007, 7, Appearance → "Labeled"},
{{kD, 0.2, "$k_D$"}, 0.002, 2, Appearance → "Labeled"},
{{kon, 0.7, "$k_{on}$"}, .007, 7, Appearance → "Labeled"},
{{koff, 0.01, "$k_{off}$"}, 0.01, 1, Appearance → "Labeled"},
{{Tcell0, 1, "Tcell"}, 0, 10, Appearance → "Labeled"},
{{CD190, 1, "CD19"}, 0, 10, Appearance → "Labeled"},
{{complex0, 0, "complex"}, 0, 10, Appearance → "Labeled"}]
```

Out[75]=



Cell Count Raw Data

Filename, M1	BV173-CD19+, # of Events	T cell-CD3+, # of Events	Tcell-CD8+, # of Events	Tcell-CD4+, # of Events	Day	CONDITION	CD19+	CD3+
A06 day1-1-BV173.fcs compensated	123433	8	0	8	0	BV173	10000	
A07 day1-2-BV173.fcs compensated	152375	25	0	25	1	BV173	137904	
A08 day1-1-PBMC.fcs compensated	226	9890	9841	49	2	BV173	187940.5	
A09 day1-2-PBMC.fcs compensated	20	9528	9486	42	3	BV173	327563.5	
A10 day1-1-PBMC+BV173.fcs compensated	143888	7916	7876	40	4	BV173	378432	
A11 day1-2-PBMC+BV173.fcs compensated	118121	9259	9215	44	5	BV173	473459	
A12 day1-1-PBMC+BV173+B.fcs compensated	10533	8124	8121	3				
B01 day1-2-PBMC+BV173+B.fcs compensated	7912	5344	5340	4	0	CD3+		5000
B02 day1-1-PBMC+BV173+B+N.fcs compensat	5919	9199	9196	3	1	CD3+		9709
B03 day1-2-PBMC+BV173+B+N.fcs compensat	7369	8615	8611	4	2	CD3+		8382
B04 day1-1-PBMC+BV173+B+D.fcs compensat	27971	5869	5864	5	3	CD3+		9207.5
B05 day1-2-PBMC+BV173+B+D.fcs compensat	64647	6254	6249	5	4	CD3+		9589
B06 day2-1-BV173.fcs compensated	217289	4	1	3	5	CD3+		8190
B07 day2-2-BV173.fcs compensated	158592	4	0	4				
B08 day2-1-PBMC.fcs compensated	34	8170	7134	1036	0	BV173+CD3+		
B09 day2-2-PBMC.fcs compensated	9	8594	7544	1050	1	BV173+CD3+	131004.5	8587.5
B10 day2-1-BV173+PBMC.fcs compensated	226177	3904	3849	55	2	BV173+CD3+	242334	4004
B11 day2-2-BV173+PBMC.fcs compensated	258491	4104	4071	33	3	BV173+CD3+	344659	2460
B12 day2-1-PBMC+BV173+B.fcs compensated	482	12039	12039	0	4	BV173+CD3+	283220.5	608.5
C01 day2-2-PBMC+BV173+B.fcs compensated	894	13613	13610	3	5	BV173+CD3+	283220.5	633
C02 day2-1-PBMC+BV173+B+N.fcs compensat	809	16437	16435	2				
C03 day2-2-PBMC+BV173+B+N.fcs compensat	902	12806	12805	1	0	BV173+CD3+B	10000	5000
C04 day2-2-PBMC+BV173+B+D.fcs compensat	35897	3597	3594	3	1	BV173+CD3+B	9222.5	6734
C05 day2-2-PBMC+BV173+B+D.fcs compensat	53710	5680	5654	26	2	BV173+CD3+B	688	12826
C06 day3-1-BV173.fcs compensated	329590	21	1	20	3	BV173+CD3+B	502.5	34006
C07 day3-2-BV173.fcs compensated	325537	8	0	8	4	BV173+CD3+B	4996	26975.5
C08 day3-1-PBMC.fcs compensated	280	9273	2220	7053	5	BV173+CD3+B	1985	15307
C09 day3-2-PBMC.fcs compensated	11	9142	3064	6078				
C10 day3-1-PBMC+BV173.fcs compensated	348732	2429	2352	77				
C11 day3-2-PBMC+BV173.fcs compensated	340586	2491	2422	69	0	BV173+CD3+B+N	10000	5000
C12 day3-1-PBMC+BV173+B.fcs compensated	554	32296	32284	12	1	BV173+CD3+B+N	6644	8907
D01 day3-2-PBMC+BV173+B.fcs compensated	451	35716	35696	20	2	BV173+CD3+B+N	855.5	14621.5
D02 day3-1-PBMC+BV173+B+N.fcs compensat	443	35269	35257	12	3	BV173+CD3+B+N	481.5	38847
D03 day3-2-PBMC+BV173+B+N.fcs compensat	520	42425	42407	18	4	BV173+CD3+B+N	1145	38847
D04 day3-1-PBMC+BV173+B+D.fcs compensat	42877	2923	2916	7	5	BV173+CD3+B+N	1152	10849
D05 day3-2-PBMC+BV173+B+D.fcs compensat	39036	3728	3724	4				
D06 day4-1-BV173.fcs compensated	371570	21	1	20	0	BV173+CD3+B+D	10000	5000
D07 day4-2-BV173.fcs compensated	385294	15	1	14	1	BV173+CD3+B+D	46309	6061.5
D08 day4-1-PBMC.fcs compensated	115	9819	1702	8117	2	BV173+CD3+B+D	62752	4638.5
D09 day4-2-PBMC.fcs compensated	5	9359	1252	8107	3	BV173+CD3+B+D	40956.5	3325.5
D10 day4-1-BV173+PBMC.fcs compensated	302538	659	562	97	4	BV173+CD3+B+D	24776	1716
D11 day4-2-BV173+PBMC.fcs compensated	263903	558	456	102	5	BV173+CD3+B+D	15901	543.5
D12 day4-1-BV173+PBMC+B.fcs compensated	695	29418	29399	19				
E01 day4-2-BV173+PBMC+B.fcs compensated	9297	24533	24417	116				
E02 day4-1-BV173+PBMC+B+N.fcs compensat	1250	10697	10686	11				
E03 day4-2-BV173+PBMC+B+N.fcs compensat	1040	17686	17665	21				
E04 day4-1-BV173+PBMC+B+D.fcs compensat	25214	1267	1259	8				
E05 day4-2-BV173+PBMC+B+D.fcs compensat	24338	2165	2156	9				
E06 day5-BV173-1.fcs compensated	469679	17	2	15				
E07 day5-BV173-2.fcs compensated	477239	22	1	21				
E08 day5-PBMC-1.fcs compensated	59	8346	1161	7185				
E09 day5-PBMC-2.fcs compensated	16	8034	836	7198				
E10 DAY5-1-BV+PBMC.fcs compensated	160615	459	343	116				
E11 DAY5-2-BV+PBMC.fcs compensated	162864	807	646	161				
E12 DAY5-1-BV+PBMC+B.fcs compensated	2028	13619	13573	46				
F01 DAY5-2-BV+PBMC+B.fcs compensated	1942	16995	16966	29				
F02 DAY5-1-BV+PBMC+B+N.fcs compensated	850	11383	11343	40				
F03 DAY5-2-BV+PBMC+B+N.fcs compensated	1454	10315	10256	59				
F04 DAY5-1-BV+PBMC+B+D.fcs compensated	18656	488	480	8				
F05 DAY5-2-BV+PBMC+B+D.fcs compensated	13146	599	595	4				



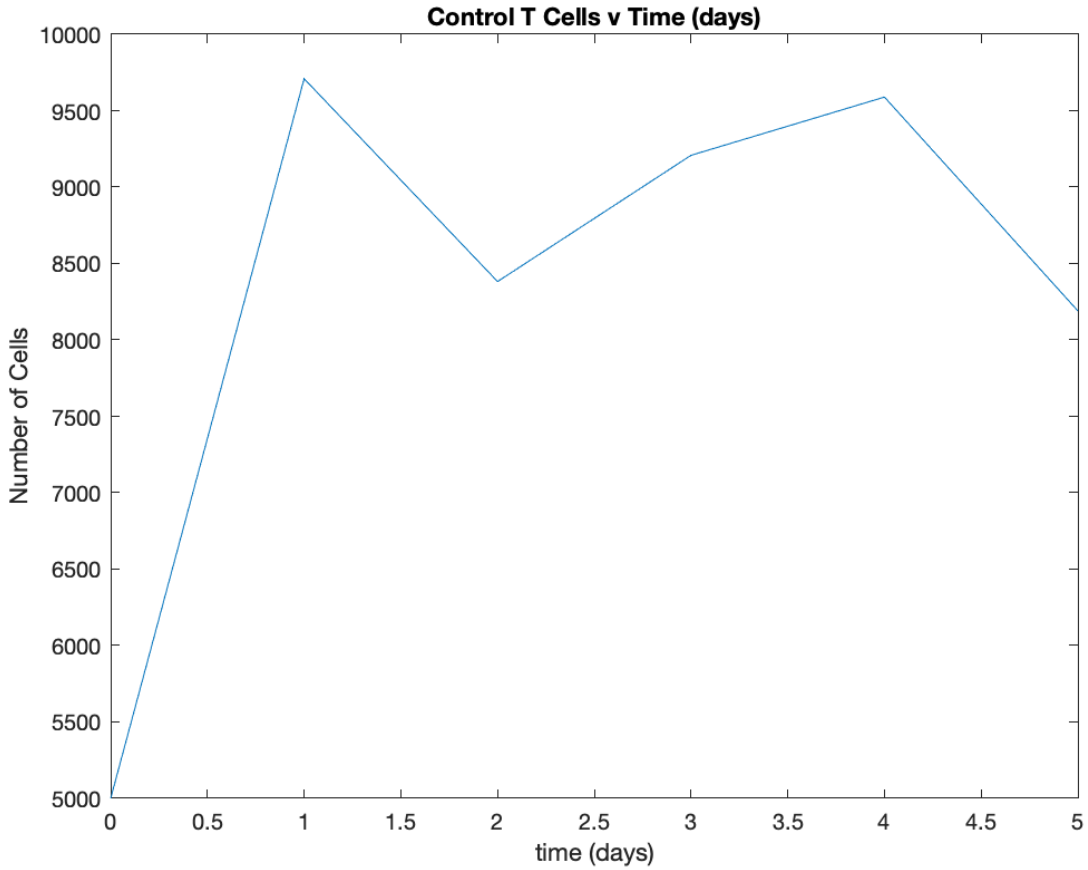
Other method of representing *in vitro* data by plotting each condition together for T cells and B cells. This shows the effects of combination therapy on PBMC derived T cells and BV173 B cells that were cocultured together as described in the methods section. (A) T cells with no treatment were measured over 5 days. (B) The effect of therapy with blinatumomab (blue), blinatumomab and nilotinib (orange), and blinatumomab and dasatinib (grey) on T cells was measured over 5 days. (C) B cells with no treatment were measured over 5 days. (D) The effect of therapy with blinatumomab (blue), blinatumomab and nilotinib (orange), and blinatumomab and dasatinib (grey) on B cells was measured over 5 days.

Using MLE to fit Poisson Distribution to Data

```
clc
clear
```

CONTROL

```
time=[0 1 2 3 4 5];
T_data_c=[5000 9709 8382 9208 9589 8190] ; %rounded to integer
B_data_c=[10000 137904 187941 327564 378432 473459]; %rounded to integer
figure
plot(time, T_data_c)
xlabel("time (days)")
ylabel("Number of Cells")
title("Control T Cells v Time (days)")
```



```
figure
plot(time, B_data_c)
xlabel("time (days)")
ylabel("Number of Cells")
title("Control B Cells v Time (days)")
```

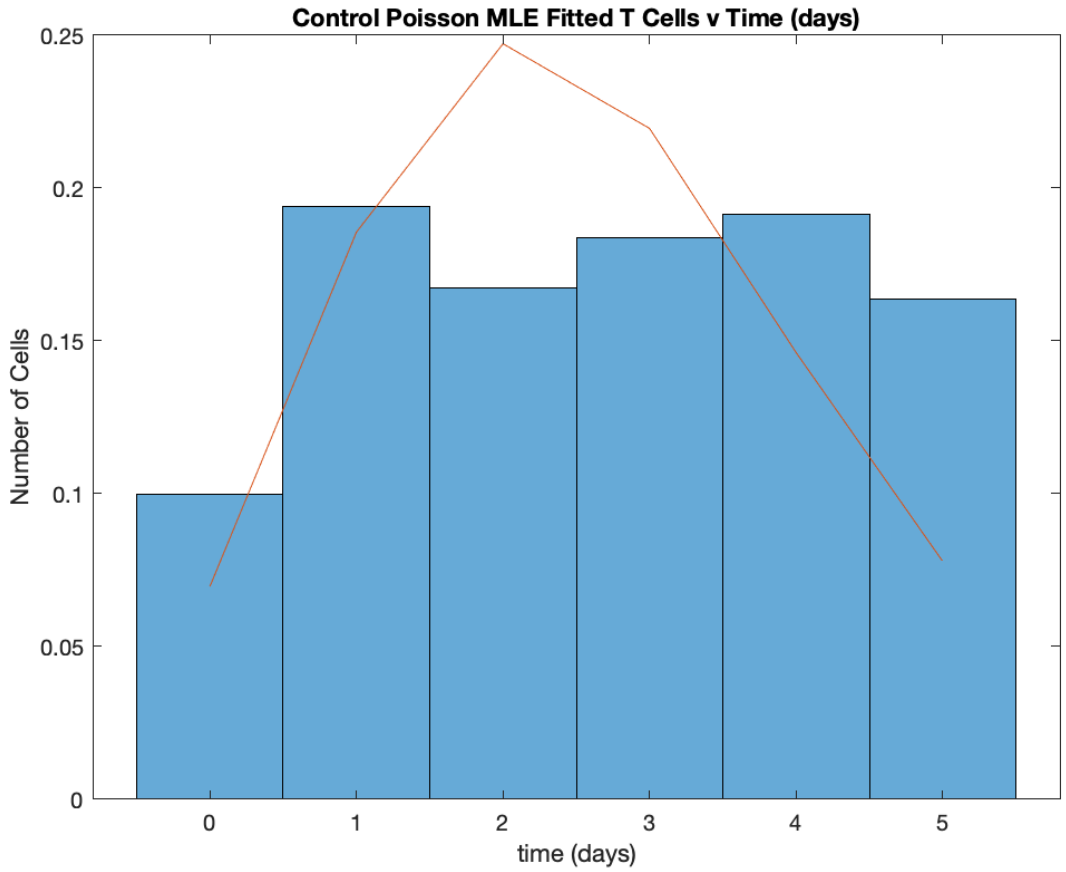
now lets try to fit using MLE and Poisson Dist for T cell data

```
%cleaning up data into correct feed in format
Zero=repmat(0,1,T_data_c(1));
One=repmat(1,1,T_data_c(2));
Two=repmat(2,1,T_data_c(3));
Three=repmat(3,1,T_data_c(4));
Four=repmat(4,1,T_data_c(5));
Five=repmat(5,1,T_data_c(6));
data1_T=[Zero One Two Three Four Five];
[phat_T,pci_T] = mle(data1_T, 'Distribution','Poisson') % gives lambda and confidence
intervals
```

```
phat_T = 2.6639
pci_T = 2×1
    2.6496
    2.6782
```

```
x = [0 1 2 3 4 5];
lambda_T=phat_T;
y_T = pdf('Poisson',x,lambda_T);
figure
histogram(data1_T,'Normalization','pdf')   %plotting data with normalization
hold on
```

```
plot(x,y_T)% plotting fitted data
hold off
xlabel("time (days)")
ylabel("Number of Cells")
title("Control Poisson MLE Fitted T Cells v Time (days)")
```



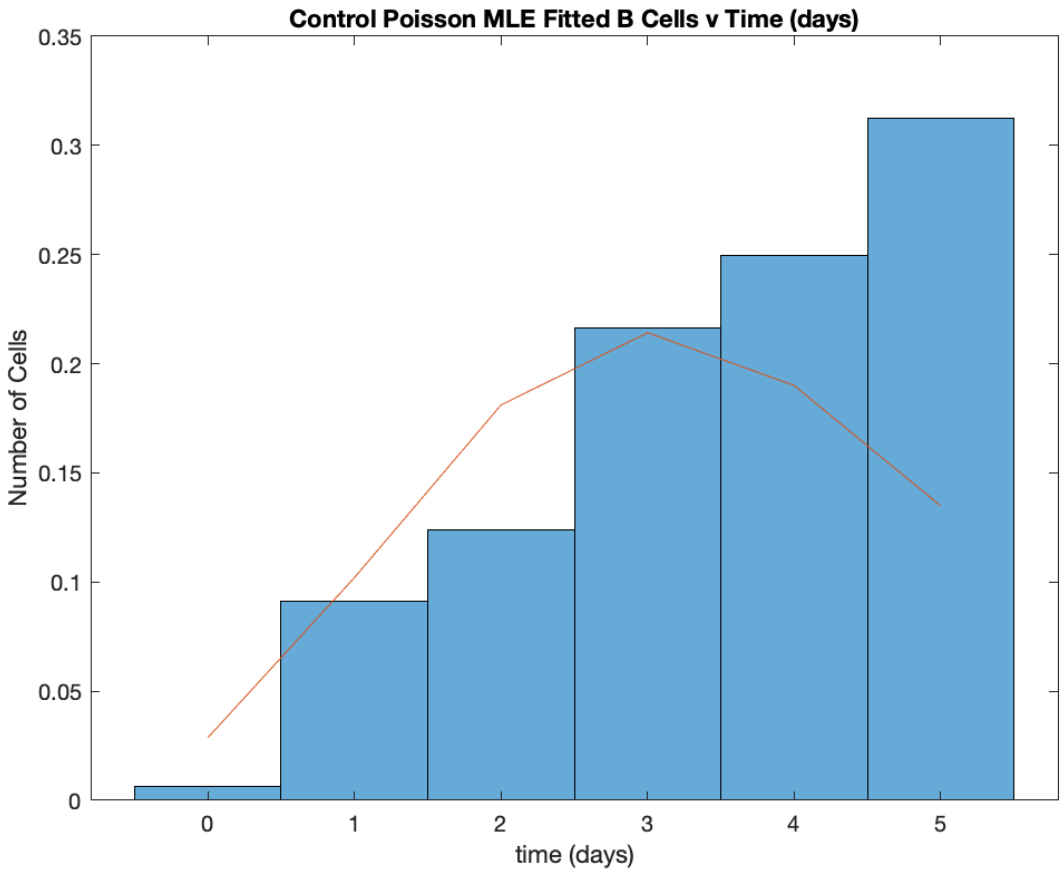
now lets try to fit using MLE and Poisson Dist for B cell data

```
%cleaning up data into correct feed in format
Zero_B=repmat(0,1,B_data_c(1));
One_B=repmat(1,1,B_data_c(2));
Two_B=repmat(2,1,B_data_c(3));
Three_B=repmat(3,1,B_data_c(4));
Four_B=repmat(4,1,B_data_c(5));
```

```
Five_B=repmat(5,1,B_data_c(6));
data1_B=[Zero_B One_B Two_B Three_B Four_B Five_B];
[phat_B,pci_B] = mle(data1_B, 'Distribution','Poisson') % gives lambda and confidence intervals
```

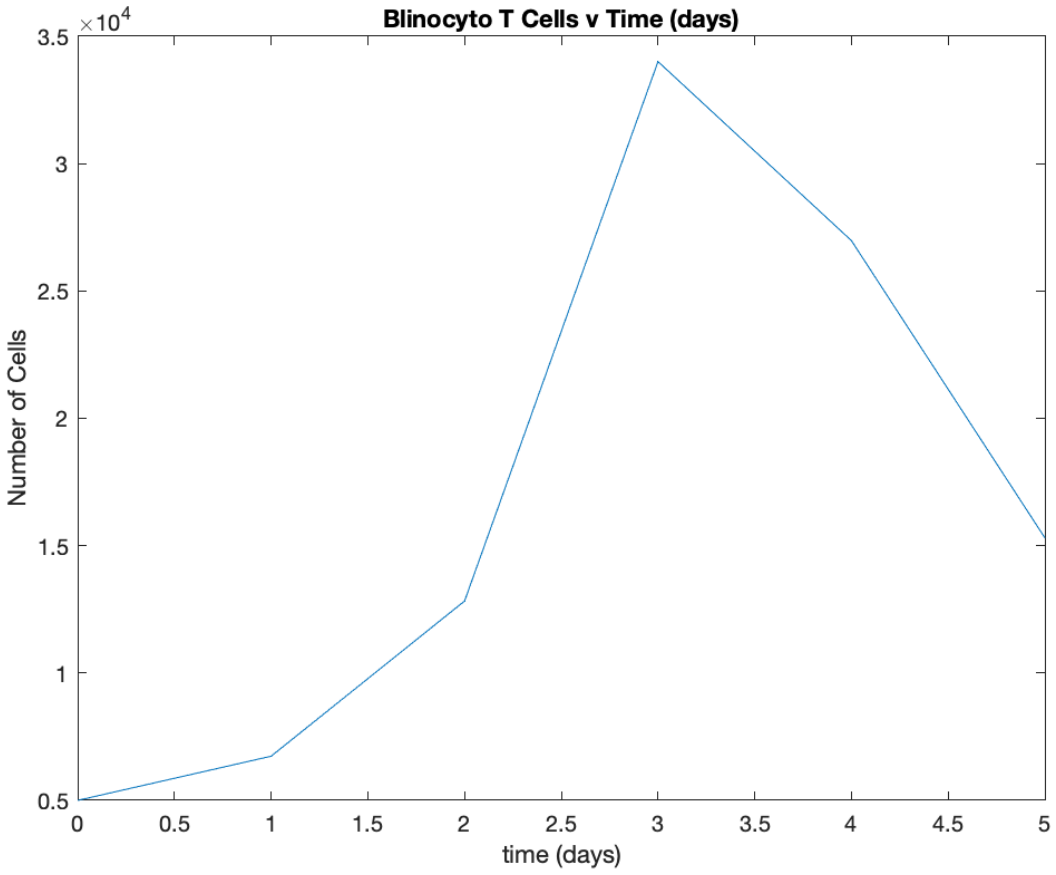
```
phat_B = 3.5488
pci_B = 2×1
    3.5458
    3.5518
```

```
x = [0 1 2 3 4 5];
lambda_B=phat_B;
y_B = pdf('Poisson',x,lambda_B);
figure
histogram(data1_B,'Normalization','pdf')   %plotting data with normalization
hold on
plot(x,y_B)% plotting fitted data
hold off
xlabel("time (days)")
ylabel("Number of Cells")
title("Control Poisson MLE Fitted B Cells v Time (days)")
```

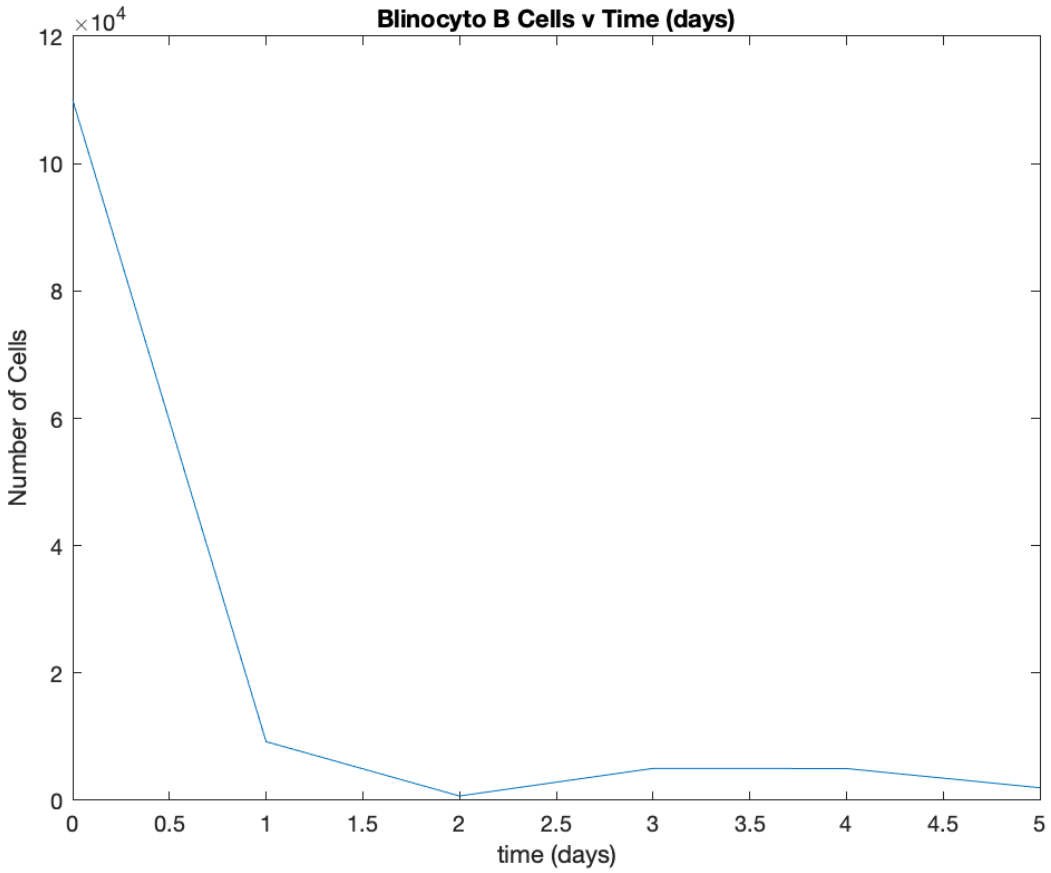


Blinocyto Data

```
time=[0 1 2 3 4 5];
T_data_b=[5000 6734 12826 34006        26976 15307] ; %rounded to integer
B_data_b=[110000 9223 688 5023 4996 1985]; %rounded to integer
figure
plot(time, T_data_b)
xlabel("time (days)")
ylabel("Number of Cells")
title("Blinocyto T Cells v Time (days)")
```




```
figure
plot(time, B_data_b)
xlabel("time (days)")
ylabel("Number of Cells")
title("Blinocyto B Cells v Time (days)")
```



now lets try to fit using MLE and Poisson Dist for T cell data

```
%cleaning up data into correct feed in format
Zero_b=repmat(0,1,T_data_b(1));
One_b=repmat(1,1,T_data_b(2));
Two_b=repmat(2,1,T_data_b(3));
Three_b=repmat(3,1,T_data_b(4));
Four_b=repmat(4,1,T_data_b(5));
Five_b=repmat(5,1,T_data_b(6));
data1_Tb=[Zero_b One_b Two_b Three_b Four_b Five_b];
[phat_Tb,pci_Tb] = mle(data1_Tb, 'Distribution','Poisson') % gives lambda and
confidence intervals
```

```
phat_Tb = 3.1616
```

```
pci_Tb = 2×1
    3.1506
    3.1726
```

```
x = [0 1 2 3 4 5];
lambda_Tb=phat_Tb;
y_Tb = pdf('Poisson',x,lambda_Tb);
figure
histogram(data1_Tb,'Normalization','pdf')   %plotting data with normalization
hold on
plot(x,y_Tb)% plotting fitted data
hold off
xlabel("time (days)")
ylabel("Number of Cells")
title("Blinicyto Poisson MLE Fitted T Cells v Time (days)")
```

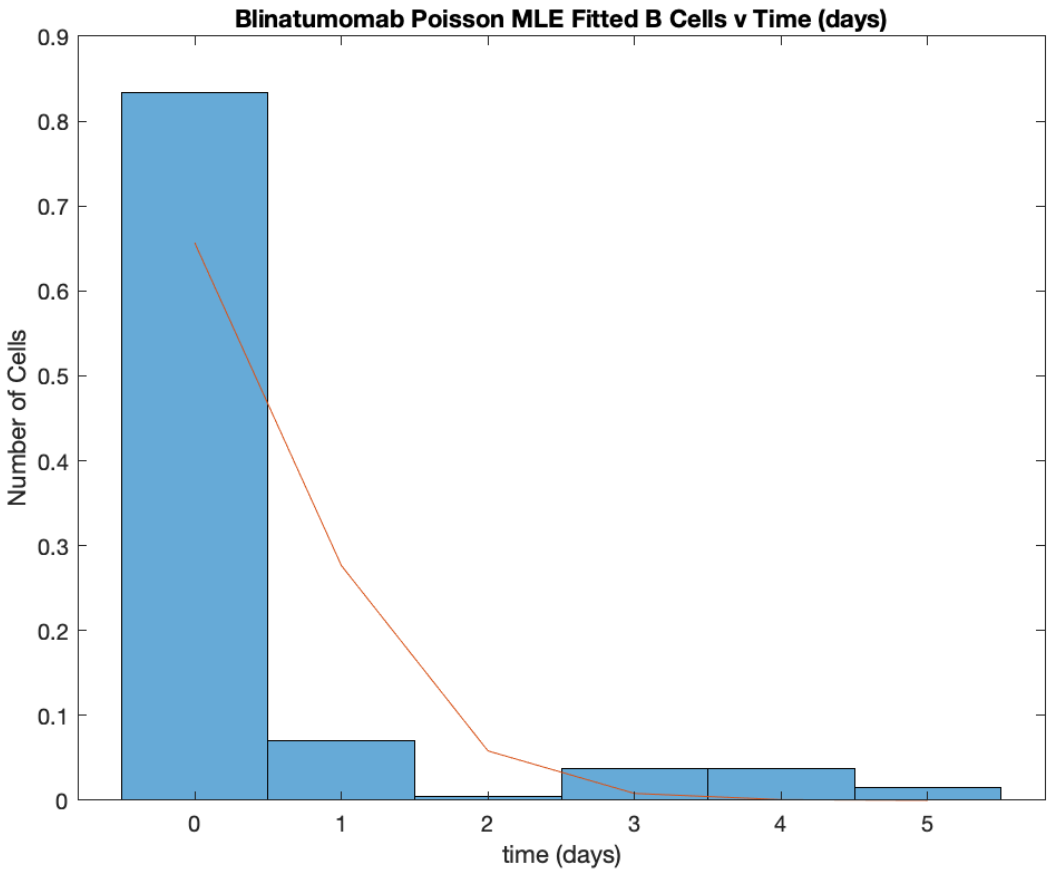
now lets try to fit using MLE and Poisson Dist for B cell data

```
%cleaning up data into correct feed in format
Zero_Bb=repmat(0,1,B_data_b(1));
One_Bb=repmat(1,1,B_data_b(2));
Two_Bb=repmat(2,1,B_data_b(3));
Three_Bb=repmat(3,1,B_data_b(4));
Four_Bb=repmat(4,1,B_data_b(5));
Five_Bb=repmat(5,1,B_data_b(6));
data1_Bb=[Zero_Bb One_Bb Two_Bb Three_Bb Four_Bb Five_Bb];
[phat_Bb,pci_Bb] = mle(data1_Bb, 'Distribution','Poisson') % gives lambda and
confidence intervals
```

```
phat_Bb = 0.4213
```

```
pci_Bb = 2×1
    0.4178
    0.4248
```

```
x = [0 1 2 3 4 5];
lambda_Bb=phat_Bb;
y_Bb = pdf('Poisson',x,lambda_Bb);
figure
histogram(data1_Bb,'Normalization','pdf')   %plotting data with normalization
hold on
plot(x,y_Bb)% plotting fitted data
hold off
xlabel("time (days)")
ylabel("Number of Cells")
title("Blinatumomab Poisson MLE Fitted B Cells v Time (days)")
```



Blinocyto + Nilotinib Data

```
time=[0 1 2 3 4 5];
T_data_bn=[5000 8907 14622 38847 38847 10849] ; %rounded to integer
B_data_bn=[10000 6644 856 482 1145 1152]; %rounded to integer
figure
plot(time, T_data_bn)
xlabel("time (days)")
ylabel("Number of Cells")
title("Blinatumomab + Nilotinib T Cells v Time (days)")
```

```
figure
plot(time, B_data_bn)
```

```
xlabel("time (days)")
ylabel("Number of Cells")
title("Blinatumomab + Nilotinib B Cells v Time (days)")
```

now lets try to fit using MLE and Poisson Dist for T cell data

```
%cleaning up data into correct feed in format
Zero_bn=repmat(0,1,T_data_bn(1));
One_bn=repmat(1,1,T_data_bn(2));
Two_bn=repmat(2,1,T_data_bn(3));
Three_bn=repmat(3,1,T_data_bn(4));
Four_bn=repmat(4,1,T_data_bn(5));
Five_bn=repmat(5,1,T_data_bn(6));
data1_Tbn=[Zero_bn One_bn Two_bn Three_bn Four_bn Five_bn];
[phat_Tbn,pci_Tbn] = mle(data1_Tbn, 'Distribution','Poisson') % gives lambda and
confidence intervals
```

```
phat_Tbn = 3.1120
pci_Tbn = 2×1
    3.1019
    3.1221
```

```
x = [0 1 2 3 4 5];
lambda_Tbn=phat_Tbn;
y_Tbn = pdf('Poisson',x,lambda_Tbn);
figure
histogram(data1_Tbn,'Normalization','pdf')   %plotting data with normalization
hold on
plot(x,y_Tbn)% plotting fitted data
hold off
```

```
xlabel("time (days)")
ylabel("Number of Cells")
title("Blinatumomab + Nilotinib Poisson MLE Fitted T Cells v Time (days)")
```

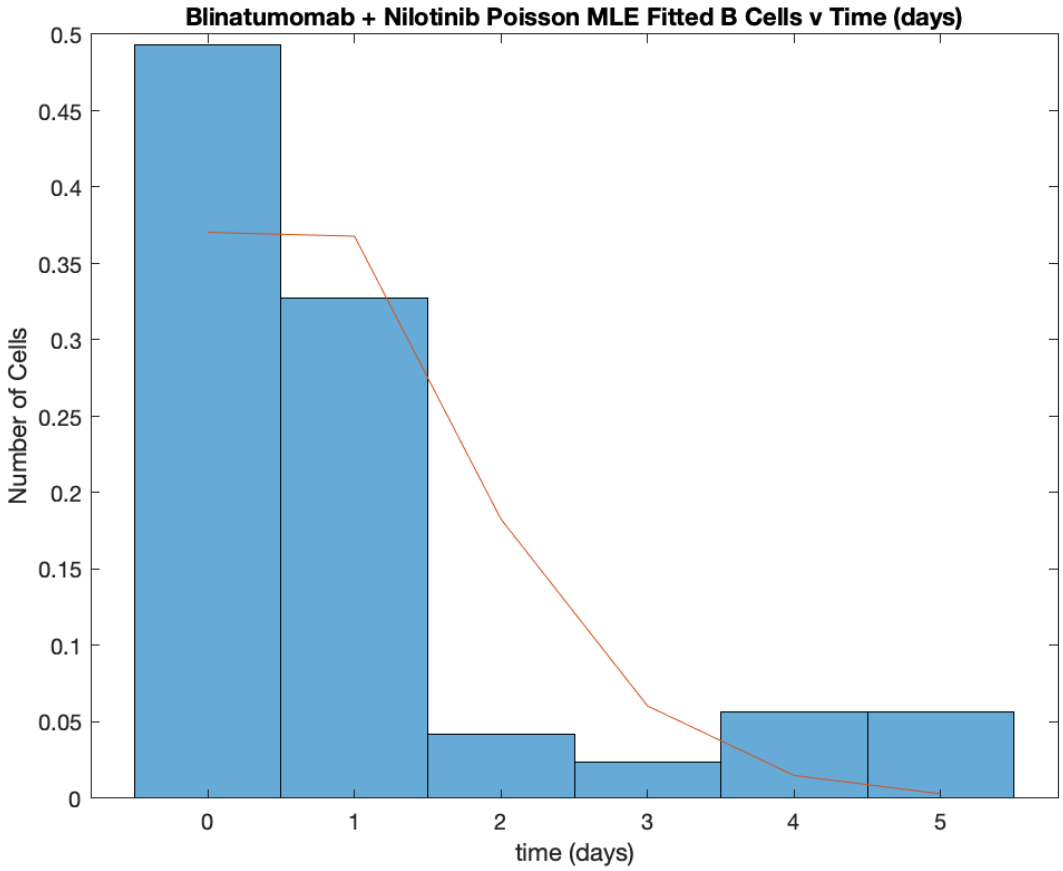
now lets try to fit using MLE and Poisson Dist for B cell data

```
%cleaning up data into correct feed in format
Zero_Bbn=repmat(0,1,B_data_bn(1));
One_Bbn=repmat(1,1,B_data_bn(2));
Two_Bbn=repmat(2,1,B_data_bn(3));
Three_Bbn=repmat(3,1,B_data_bn(4));
Four_Bbn=repmat(4,1,B_data_bn(5));
Five_Bbn=repmat(5,1,B_data_bn(6));
data1_Bbn=[Zero_Bbn One_Bbn Two_Bbn Three_Bbn Four_Bbn Five_Bbn];
[phat_Bbn,pci_Bbn] = mle(data1_Bbn, 'Distribution','Poisson') % gives lambda and
confidence intervals
```

```
phat_Bbn = 0.9932
pci_Bbn = 2×1
    0.9795
    1.0070
```

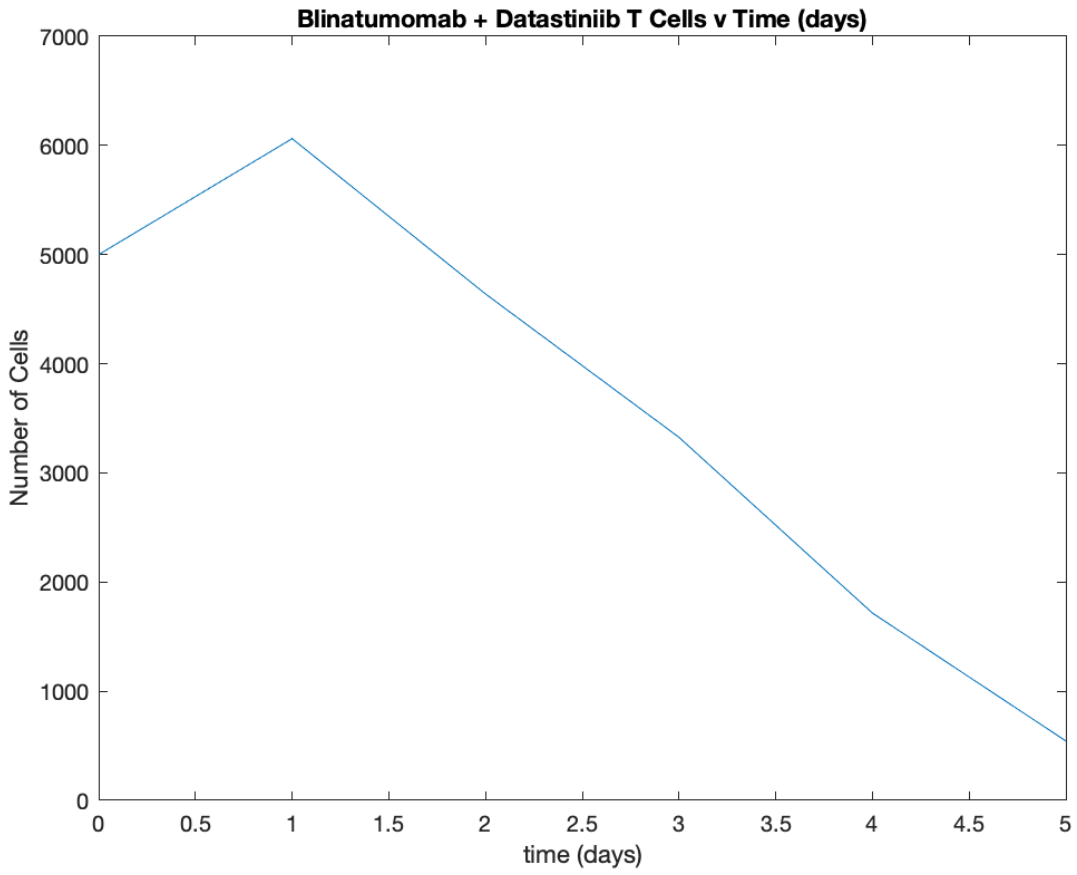
```
x = [0 1 2 3 4 5];
lambda_Bbn=phat_Bbn;
y_Bbn = pdf('Poisson',x,lambda_Bbn);
figure
histogram(data1_Bbn,'Normalization','pdf')   %plotting data with normalization
hold on
plot(x,y_Bbn)% plotting fitted data
hold off
```

```
xlabel("time (days)")
ylabel("Number of Cells")
title("Blinatumomab + Nilotinib Poisson MLE Fitted B Cells v Time (days)")
```



Blinatumomab + Datastiniib Data

```
time=[0 1 2 3 4 5];
T_data_bd=[5000 6062 4639 3326 1716 544] ; %rounded to integer
B_data_bd=[10000 46309 62752 40957 24776 15901]; %rounded to integer
figure
plot(time, T_data_bd)
xlabel("time (days)")
ylabel("Number of Cells")
title("Blinatumomab + Datastiniib T Cells v Time (days)")
```




```
figure
plot(time, B_data_bd)
xlabel("time (days)")
ylabel("Number of Cells")
title("Blinatumomab + Datastiniib B Cells v Time (days)")
```

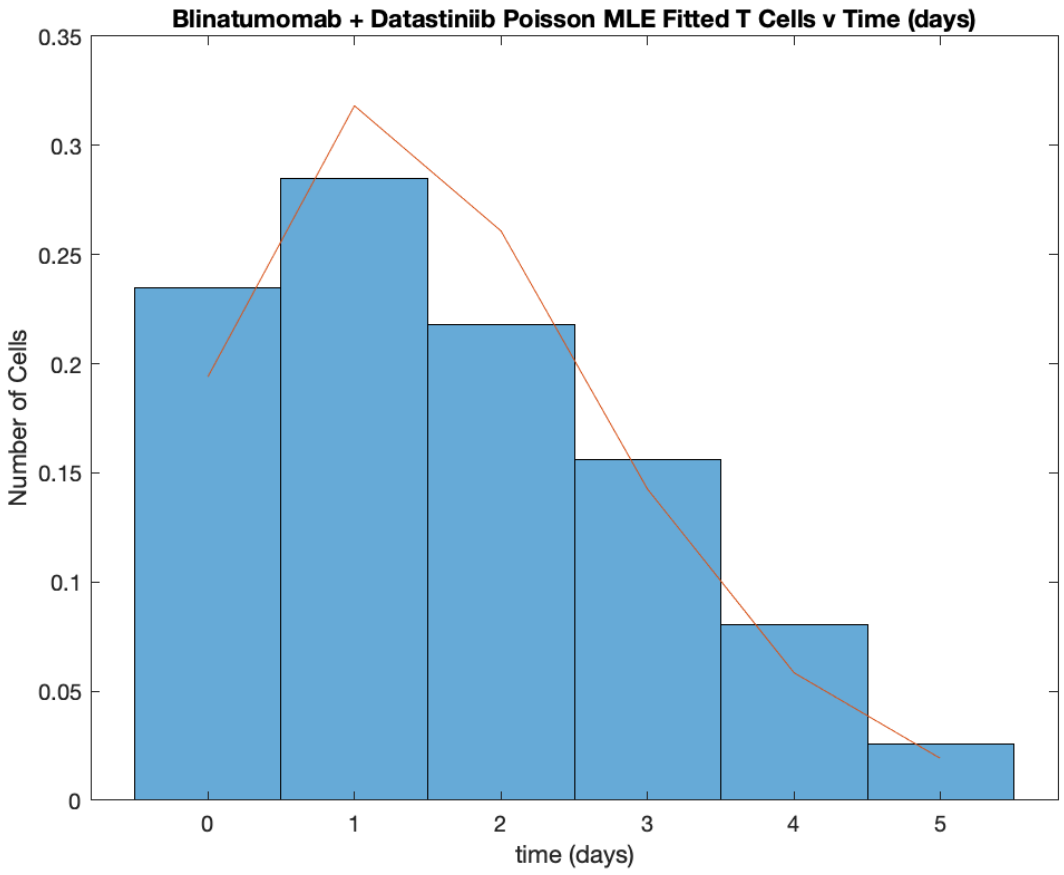
now lets try to fit using MLE and Poisson Dist for T cell data

```
%cleaning up data into correct feed in format
Zero_bd=repmat(0,1,T_data_bd(1));
One_bd=repmat(1,1,T_data_bd(2));
Two_bd=repmat(2,1,T_data_bd(3));
Three_bd=repmat(3,1,T_data_bd(4));
```

```
Four_bd=repmat(4,1,T_data_bd(5));
Five_bd=repmat(5,1,T_data_bd(6));
data1_Tbd=[Zero_bd One_bd Two_bd Three_bd Four_bd Five_bd];
[phat_Tbd,pci_Tbd] = mle(data1_Tbd, 'Distribution','Poisson') % gives lambda and
confidence intervals
```

```
phat_Tbd = 1.6396
pci_Tbd = 2×1
    1.6224
    1.6568
```

```
x = [0 1 2 3 4 5];
lambda_Tbd=phat_Tbd;
y_Tbd = pdf('Poisson',x,lambda_Tbd);
figure
histogram(data1_Tbd,'Normalization','pdf')   %plotting data with normalization
hold on
plot(x,y_Tbd)% plotting fitted data
hold off
xlabel("time (days)")
ylabel("Number of Cells")
title("Blinatumomab + Datastiniib Poisson MLE Fitted T Cells v Time (days)")
```



now lets try to fit using MLE and Poisson Dist for B cell data

```
%cleaning up data into correct feed in format
Zero_Bbd=repmat(0,1,B_data_bd(1));
One_Bbd=repmat(1,1,B_data_bd(2));
Two_Bbd=repmat(2,1,B_data_bd(3));
Three_Bbd=repmat(3,1,B_data_bd(4));
Four_Bbd=repmat(4,1,B_data_bd(5));
Five_Bbd=repmat(5,1,B_data_bd(6));
data1_Bbd=[Zero_Bbd One_Bbd Two_Bbd Three_Bbd Four_Bbd Five_Bbd];
[phat_Bbd,pci_Bbd] = mle(data1_Bbd, 'Distribution','Poisson') % gives lambda and
confidence intervals
```

phat_Bbd = 2.3583

```
pci_Bbd = 2×1
   2.3516
   2.3650
```

```
x = [0 1 2 3 4 5];
lambda_Bbd=phat_Bbd;
y_Bbd = pdf('Poisson',x,lambda_Bbd);
figure
histogram(data1_Bbd,'Normalization','pdf')   %plotting data with normalization
hold on
plot(x,y_Bbd)% plotting fitted data
hold off
xlabel("time (days)")
ylabel("Number of Cells")
title("Blinatumomab + Datastiniib Poisson MLE Fitted B Cells v Time (days)")
```

Plotting Nicer Graphs To Compare Fitted Curves

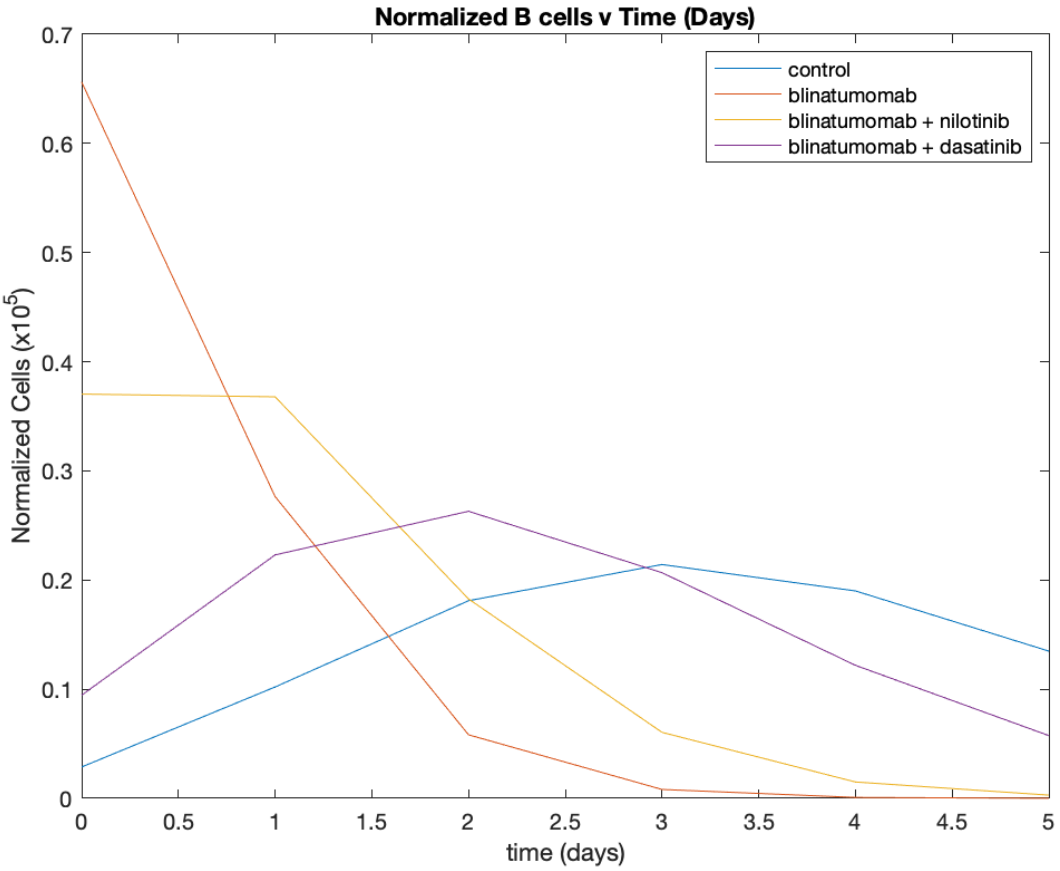
All T cell Data

```
figure
plot(x, y_T, x, y_Tb, x, y_Tbn, x, y_Tbd)
legend('control', 'blinatumomab', 'blinatumomab + nilotinib', 'blinatumomab + dasatinib')
xlabel("time (days)")
ylabel("Normalized Cells (x10^5)")
title("Normalized T cells v Time (Days)")
```

All B cell Data

```
figure
plot(x, y_B, x, y_Bb, x, y_Bbn, x, y_Bbd)
legend('control', 'blinatumomab', 'blinatumomab + nilotinib', 'blinatumomab + dasatinib')
```

```
xlabel("time (days)")
ylabel("Normalized Cells (x10^5)")
title("Normalized B cells v Time (Days)")
```



IL2 rescue western blot, BCA, Calculations ETC

Drug	Stock	Dilution 1	new Concentration	Dilution 2	new concentration	amount added to experiment	final concentration in 1 mL	Desired

Ponatanib Dilution	10mM	5uL in 495uL	100uM	10uL stock + 990uL RPMI	1 uM	40ul	40nM	40nM
Dasatinib Dilution	10mM	5uL in 495uL	100uM	10uL stock + 990uL RPMI	1 uM	10ul	10nM	10nM
Nilotinib Dilution	10mM	5uL in 495uL	100uM	10uL stock + 990uL RPMI	1 uM	130ul	130nM	130nM
Imatinib Dilution	10mM	5uL in 495uL	100uM	10uL stock + 990uL RPMI	1 uM	450ul	450nM	450nM
IL 2	0.5 mg in 1 mL	5 uL in 0.5mL	5,000 ng/mL	200uL in 1mL	1,000 ng/mL	100uL	100 ng/mL	100 ng/mL

Condition	Ponatinib	Dasatinib	Nilotinib	Imatinib	diluted Beads	IL2 (100ng)	CELLS(2*10^6)	serum free RPMI
C-	0	0	0	0	0	0	200	800
C+	0	0	0	0	50	0	200	750
P+	40	0	0	0	50	0	200	710
D+	0	10	0	0	50	0	200	740
N+	0	0	130	0	50	0	200	620
I+	0	0	0	450	50	0	200	300
C- + IL2	0	0	0	0	0	10	200	790
C+IL2	0	0	0	0	50	10	200	740
P+IL2	40	0	0	0	50	10	200	700

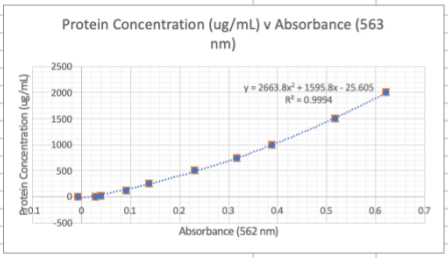
D+ IL2	0	10	0	0	50	10	200	730
N+IL2	0	0	130	0	50	10	200	610
I+IL2	0	0	0	450	50	10	200	290

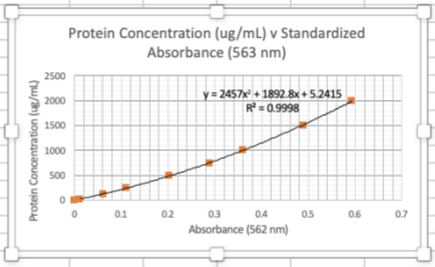
								western 3_time		
Sample	Protein Concentration (ug/mL)	Adjusted Protein Concentration (ug/uL)	w/ 25uL SB	Sample (uL)	1x SB (uL)	Volume Total (uL)	ug in 20uL	sample	1X	ul in total
C-	1209.980672	1.209980672	1.008317227	19.9	0.1	20	10.03275641	39.8	0.2	40
C+	1387.53405	1.38753405	1.156278375	17.3	2.7	20	10.00180794	34.6	5.4	40
C-IL2	1535.56329	1.53556329	1.279636075	15.7	4.3	20	10.04514319	31.4	8.6	40
C+ IL2	1544.20393	1.54420393	1.286836608	15.6	4.4	20	10.03732555	31.2	8.8	40
P+	1676.17213	1.67617213	1.396810108	14.4	5.6	20	10.05703278	28.8	11.2	40
P+ IL2	1805.63835	1.80563835	1.504698625	13.3	6.7	20	10.00624586	26.6	13.4	40
D+	1479.87783	1.47987783	1.233231917	16.3	3.7	20	10.05084012	32.6	7.4	40
D+ IL2	1399.98964	1.39998964	1.166655333	17.2	2.8	20	10.03323587	34.4	5.6	40
I+	1397.90793	1.39790793	1.164923275	17.2	2.8	20	10.01834017	34.4	5.6	40
I+ IL2	1301.60593	1.30160593	1.084671608	18.5	1.5	20	10.03321238	37	3	40
N+	1671.70193	1.67170193	1.393084942	14.4	5.6	20	10.03021158	28.8	11.2	40
N+ IL2	1658.32084	1.65832084	1.381934033	14.5	5.5	20	10.01902174	29	11	40

Sample	Protein Concentration (ug/mL)	Adjusted Protein Concentration (ug/uL)	w/ 25uL SB	Sample (uL)	1xSB (uL)	Volume Total (uL)	ug in 20uL	sample	1X	ul in total
C-	1209.980672	1.209980672	1.008317227	19.9	0.1	20	10.03275641	39.8	0.2	40
C+	1387.53405	1.38753405	1.156278375	17.3	2.7	20	10.00180794	34.6	5.4	40
C- IL2	1535.56329	1.53556329	1.279636075	15.7	4.3	20	10.04514319	31.4	8.6	40
C+ IL2	1544.20393	1.54420393	1.286836608	15.6	4.4	20	10.03732555	31.2	8.8	40
P+	1676.17213	1.67617213	1.396810108	14.4	5.6	20	10.05703278	28.8	11.2	40
P+ IL2	1805.63835	1.80563835	1.504698625	13.3	6.7	20	10.00624586	26.6	13.4	40
D+	1479.8783	1.4798783	1.233231917	16.3	3.7	20	10.05084012	32.6	7.4	40
D+ IL2	1399.9864	1.3999864	1.166655333	17.2	2.8	20	10.03323587	34.4	5.6	40
I+	1397.90793	1.39790793	1.164923275	17.2	2.8	20	10.01834017	34.4	5.6	40
I+ IL2	1301.60593	1.30160593	1.084671608	18.5	1.5	20	10.03321238	37	3	40
N+	1671.70193	1.67170193	1.393084942	14.4	5.6	20	10.03021158	28.8	11.2	40
N+ IL2	1658.32084	1.65832084	1.381934033	14.5	5.5	20	10.01902174	29	11	40

Results for ABS 562(1) - channel 1 (A)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.61	0.504	0.387	0.332	0.235	0.136	0.091	0.039	0.029	-0.006	-0.008	-0.007
B	0.633	0.531	0.389	0.302	0.226	0.141	0.09	0.039	0.029	-0.007	-0.016	-0.017
Absorbance	0.6215	0.5175	0.388	0.317	0.2305	0.1385	0.0905	0.039	0.029	-0.0065	-0.012	-0.012
Protein Concentration (ug/mL)	2000	1500	1000	750	500	250	125	25	0	0	0	0
Absorbance Standardized	0.5925	0.4885	0.359	0.288	0.2015	0.1095	0.0615	0.01	0			
C	0.457	0.52	0.536	0.55	0.557	0.619	0.511	0.516	0.501	0.48	0.566	0.547
D	0.429	0.454	0.508	0.498	0.551	0.546	0.507	0.464	0.478	0.452	0.54	0.553
AVG Absorbance	0.443	0.487	0.522	0.524	0.554	0.5825	0.509	0.49	0.4895	0.466	0.553	0.55
Protein Concentration (ug/mL)	1204.102486	1383.3204	1533.2455	1542.0097	1676.031	1807.7925	1476.7972	1395.9154	1393.8129	1296.498	1671.4864	1657.8845
Absorbance Standardized	0.414	0.458	0.493	0.495	0.525	0.5535	0.48	0.461	0.4605	0.437	0.524	0.521
Protein Concentration (ug/mL)	1209.980672	1387.534	1535.5633	1544.2039	1676.1721	1805.6383	1479.8783	1399.9864	1397.9079	1301.6059	1671.7019	1658.3208

Equation from standard curve for absorbance	equation -7*10^-8*(x)^2+0.0004*(x)+0.023	R^2 = 0.997
Equ for concentration	y = 2663.8x^2 + 1595.8x - 25.605	R^2 = 0.9994





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ACADEMIC VITA

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EDUCATION

Bachelor of Science in Biomedical Engineering, ABET Accredited
Schreyer Honors College
The Pennsylvania State University, University Park, PA
Anticipated Graduation: May 2022 | Dean's list: SU18, FA18, SP19, FA19, SP20, FA20, SP21, FA 21

WORK EXPERIENCE

Manufacturing Science and Technology Intern — Summer 2019
Novartis Pharmaceuticals, Morris Plains, NJ

- Studied manufacturing process of CAR-T cell therapy cancer treatment and ways to optimize the process
- Developed new procedures to ensure sterility and integrity of product with Container Closure Integrity Testing

RESEARCH EXPERIENCE

Lab Assistant — Spring 2021-Present
Dong Lab, The Pennsylvania State University, University Park, PA

- Conduct research on acute lymphoblastic leukemia and kinetic interactions with immune system and therapy intervention
- Combine dry lab skills and benchwork to design computational models and validate the models with lab obtained data

REU Lab Assistant — Summer 2021
Segura Lab, Duke University, Durham, NC

- Studied injectable hydrogels for stroke application *in vitro* and *in vivo*
- Optimized cell spreading assay and designed cell migration assay to study efficacy of gel in recruiting neural progenitor cells

Lab Assistant — 2018-2020
Rolls Lab, The Pennsylvania State University, University Park, PA

- Practiced neurobiological research on drosophila of class IV motor neurons
- Studied localization of proteins on synaptic boutons and signaling in axon terminals with regards to neurodegenerative disease

REU Lab Assistant — Summer 2020
Hogan Lab**,** Dartmouth College

- Conducted research in microbiology with bacterial fungal interactions
- Used R programming to find and quantify patterns in journals regarding bacteria and their relation to disease

LEADERSHIP

Maker Ambassadors, Penn State Learning Factory Student Outreach — 2019-Present

- Part of a team that leads BUILD Nights at the Learning Factory
- Promote inclusive environment for each build

Mentor, Penn State Women in Engineering Program Orientation — 2019-2021

- Provide yearlong support for incoming women engineers
- Organize activities to promote team bonding and aid with retention

President**,** Millennium Society — 2020-2021

Vice President of Housing, Phi Sigma Rho Engineering Sorority — 2020-2021

INVOLVEMENT

Member, Society of Women Engineers (PSU) — 2018-Present

Member, Penn State Coffee Club — 2018-Present

HONORS

Millennium Scholar — 2018-Present
ABRCMS Presenter Award — 2019
President's Freshman Award — 2018-2019
Recipient, Girl Scouts of America Gold Award — 2017