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Combinational Therapy for Acute Lymphoblastic Leukemia with Tyrosine Kinase Inhibitors and Bispecific T Cell Engagers *In Vitro*

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ABSTRACT

Acute Lymphoblastic Leukemia (ALL) is the second most common acute leukemia in adults and has a poor prognosis in most adult cases. Combinational therapies utilizing different pharmacological mechanisms have provided new momentum to developing efficient treatments for aggressive cancers such as ALL. There are two ongoing clinical trials testing combination treatments of a SRC family tyrosine kinase inhibitor (SRC TKI) with blinatumomab, a bispecific antibody engager (biTE), for adults with Philadelphia Chromosome positive (Ph+) B cell ALL. One trial is testing blinatumomab with ponatinib, and the second trial is testing blinatumomab with dasatinib. Treating ALL with a combination of a biTE and TKIs intends to initiate the immune defense by activating the T cell receptor and inhibiting the SRC family kinase activity in the B cell receptor. However, T cell activation requires kinase signaling; and thus, multikinase inhibitors antagonize the effects of biTEs. Previous studies investigating biTE and SRC TKI combinational therapies have found unfavorable multi-drug interactions in which dual SRC TKIs have an off-target effect on the efficacy of blinatumomab. Our objective was to investigate i) the biological mechanisms that cause off-target effects of TKIs on biTEs and ii) possible rescue pathways for off-target effects of TKIs on biTEs through the addition of interleukins. We found that LCK plays a pivotal role on T cell receptor signaling; however, phosphorylation of LCK on tyrosine 394 is inhibited by SRC TKIs. Results showed that combination of the SRC TKIs, such as dasatinib/ponatinib with blinatumomab, inhibits the phosphorylation of LCK on Tyrosine 394. Additionally, we found that the addition of interleukins 2, 7, and 15 to dasatinib/ponatinib with blinatumomab combination therapies show rescue of LCK through phosphorylation of JAK-STAT5 signaling. Our findings can be used to explicate the significant role of TCR in combination therapy with biTEs. Future studies can extend into testing combination therapies with other kinase inhibitors with biTEs to report TKIs that will increase the efficacy of blinatumomab and propose potential therapeutic interactions that will improve ALL patient outcomes.

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- Figure 1 obtained from Wu et al. (2015)
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Chapter 1

Introduction

Acute Lymphoblastic Leukemia (ALL) is the most common pediatric cancer [1] and the second most common acute leukemia in adults [2]. ALL represents about 20% of leukemia cases in adults, with 75% of these cases being B-cell malignancies [3]. The pathogenesis of ALL most commonly involves abnormalities in proliferation and differentiation of lymphoid cells within the bone marrow, peripheral blood, or extramedullary sites [2]. While chemotherapy treatments for ALL have increased the long-term survival rate for the pediatric population to exceed 90%, the long-term survival rate for the adult population treated with the same treatments is only 35-45% [2, 3, 4], even with the addition of hematopoietic stem-cell transplantation in many cases [1]. Adults with ALL have a higher prevalence of comorbidities and a higher frequency of adverse genetic subtypes in comparison to the pediatric population with ALL. This results in greater resistance and intolerance of chemotherapy [1], as well as high risk of relapse for adults with B-cell precursor ALL [5]. Therefore, there is the need of demonstrating the possibility of long-term survival for adults with ALL without the need for chemotherapy.

Targeted therapies for adult ALL have shown promising results in recent research and have provided momentum for immunotherapies for cancer treatment [5, 6]. Bispecific antibody engagers (biTEs) are one type of targeted therapy that researchers and medical professionals continue to investigate. BiTEs have a unique design and mechanism of action, as two monoclonal antibodies are linked together through a single polypeptide chain to bind tumor cells to T cells and activate cytotoxic T cell activity (Figure 1). Blinatumomab is a biTE that was

approved in 2014 to treat adults with relapsed or refractory B-cell ALL, and a phase three clinical trial found that treatment with blinatumomab resulted in significantly higher rates of event-free and longer overall survival in comparison to chemotherapy among adults with relapsed or refractory B-cell precursor ALL [5]. Blinatumomab works by binding CD3 on T cells to CD19 antigens on B-cell ALL to activate the cytotoxic activity of T cells to kill the tumor cells [7] (Figure 1). The T cell immune response, or T cell receptor (TCR) signaling, is activated by the binding of the T cell to the target cell and the phosphorylation of lymphocyte-specific protein tyrosine kinase (LCK), a protein belonging to the SRC family kinase [8]. Research suggests that blinatumomab activates immune memory as well, and the small size of this biTE allows for rapid tissue penetration, which is essential for efficient tumor cell killing effects [5]. However, when used as a solo agent, frequent dosing is commonly needed with this treatment [5]. Due to an increase in immune activity with treatment with biTEs, possible adverse effects of biTEs include mild inflammatory symptoms, cytokine release syndrome, and neurological events [5].

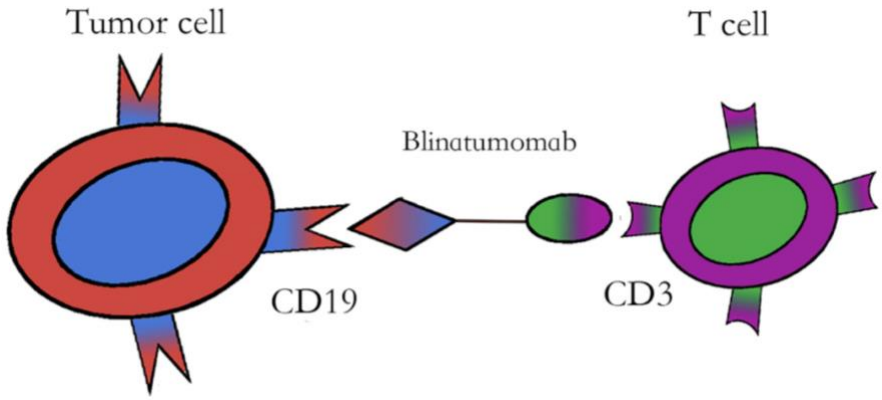


Figure 1. Mechanism of action of blinatumomab[6]

Blinatumomab binds the CD3 antigen on the T cell to the CD19 antigen on the B cell precursor ALL cells to activate the cytotoxic T cell response.

Tyrosine kinase inhibitors (TKIs) are another type of targeted therapy and is currently used to treat Philadelphia chromosome-positive (Ph+) ALL [9, 10, 11]. Ph+ ALL is characterized by

the abnormal BCR-ABL fusion oncogene [9, 11] that results from a reciprocal translocation between chromosomes 9 and 22 [12] and makes up 25% to 30% of adult ALL cases. The presence of the Philadelphia chromosome yields a worse prognosis than Ph-negative ALL, as the BCR-ABL oncogene creates an abnormal protein (a tyrosine kinase) that helps leukemia cells grow [2, 7, 9, 10, 11, 12]. With recent advances in combination treatments of chemotherapy and TKIs for Ph+ ALL, 50-70% of patients with Ph+ ALL achieve long-term survival [3, 11]. TKIs work by inhibiting the phosphorylation of the BCR-ABL oncogene in Ph+ ALL cells, preventing downstream proliferation and survival pathways of leukemic cells [13] (Figure 2) .

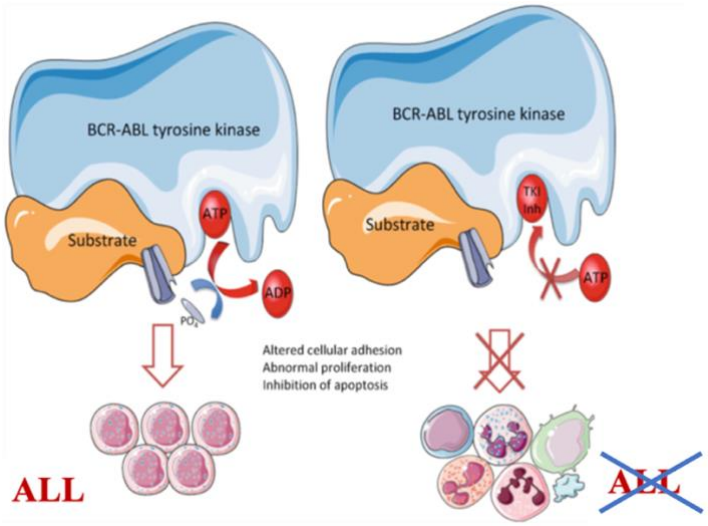


Figure 2. Mechanism of action of TKIs[13]

Right: ATP binds to ATP binding site on tumor cell to promote abnormal proliferation and inhibition of apoptosis. Left: TKIs bind to the ATP binding sites on tumor cells, preventing tumor cell proliferation and survival.

Imatinib is a first generation TKI that has increased remission rates to exceed 90% when used alone and in combination with chemotherapy as front-line therapies for Ph+ ALL [14]. Imatinib is a non-SRC TKI and prevents the proliferation of Ph+ ALL cells by directly blocking the adenosine triphosphate (ATP) binding site on the BCR-ABL1 oncogene. However, patients who do not undergo allogenic hematopoietic stem-cell transplantation typically relapse due to

subsequent mutations in the BCR-ABL tyrosine kinase domain leading to acquired resistance of TKI therapies. [15]

Nilotinib and dasatinib are second generation TKIs used to treat Ph+ ALL resistant to imatinib. Second generation TKIs are more potent drugs and show activity against most BCR-ABL tyrosine kinase domain mutations involved in imatinib resistance. Nilotinib is a non-SRC TKI and has an enhanced affinity to the BCR-ABL1 oncoprotein in comparison to imatinib. [15, 16, 17] Combination treatment of nilotinib with low intensity chemotherapy has shown an overall survival rate of 70% [17]. Dasatinib is a SRC TKI used as a single agent to treat imatinib intolerant and resistant Ph+ ALL, as this drug is believed to have the highest potential in overcoming the most imatinib-resistant kinase mutation due to its low dependency on the P-loop interaction for drug binding [12]. Dasatinib has also been used in combination with chemotherapy, which only has a 37% survival rate [18].

Ponatinib, a third generation TKI, has been used to treat Ph+ ALL adult patients resistant to or intolerant of previous TKI treatments. Ponatinib is a multi-target pan-BCR/ABL inhibitor able to overcome most kinase mutations, including the T3151 mutation[16]. A phase II study found ponatinib to be a more cost effective and successful treatment in comparison to re-induction chemotherapy for Ph+ ALL patients who were not suitable for allogenic stem cell transplantation [19]. Ponatinib has also been used in combination with intensive chemotherapy to treat Ph+ ALL, yielding a 40% one-year survival rate [20].

Since combinational treatments of TKIs and chemotherapy have shown low long-term survival rates, researchers and medical professionals are investigating novel combinational therapies with biTEs and TKIs for adult ALL. Treating ALL with a combination of a biTE and TKIs intends to initiate the immune defense by activating the T cell receptor (TCR) and

inhibiting the SRC family kinase activity in the B cell receptor (BCR) [21]. There is an ongoing phase II clinical trial (NCT02143414) that is testing dasatinib in combination with blinatumomab for adult ALL, and another ongoing phase II clinical trial (NCT03263572) that is testing ponatinib in combination with blinatumomab for adult Ph+ ALL. However, previous studies investigating TKI and blinatumomab combinational therapies *in vitro* have found unfavorable multi-drug interactions in which SRC TKIs have an off-target effect on the efficacy of blinatumomab, and thus, multikinase inhibitors such as dasatinib and ponatinib antagonize the effects of biTEs [22].

The first objective of this thesis is to investigate the synergistic and antagonistic combinations of TKIs and blinatumomab and the biological mechanisms that cause off-target effects of TKIs on biTEs. LCK is a SRC family kinase that plays a crucial role in the TCR signaling (Figure 3). While TKIs prevent proliferation and survival of tumor cells, SRC TKIs block the activity of SRC family kinases such as LCK. We intend to show that SRC TKIs such as dasatinib and ponatinib are antagonists and have off-target effects on blinatumomab through the inhibition of LCK activity (Figure 4). We also intend to show that non-SRC TKIs such as imatinib and nilotinib have synergistic effects when used in combination with blinatumomab.

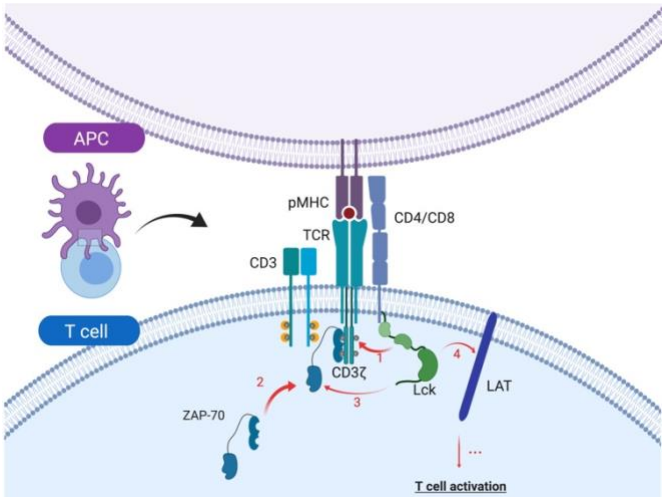


Figure 3. Role of LCK in TCR signaling[23]

LCK is recruited through interactions between T cells and antigen presenting cells (APCs; ALL cells in this study) and phosphorylates tyrosines on intracellular signaling domains, resulting in recruitment and phosphorylation of ZAP-70 and other intracellular proteins to activate cytotoxic T cell responses. Activation of cytotoxic T cell responses is essential with the use of immunotherapies to achieve long-term survival for adult ALL patients.

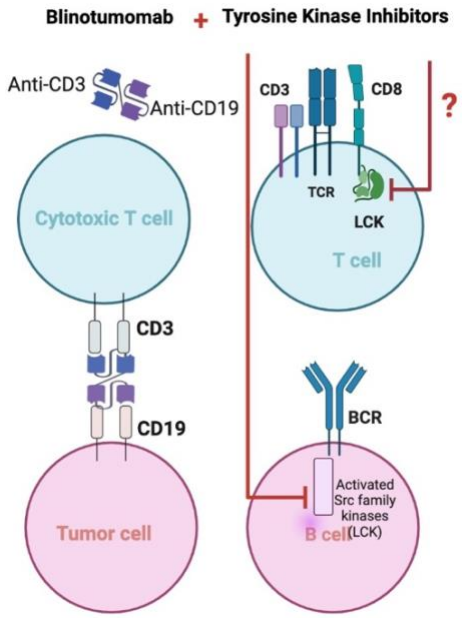


Figure 4. Hypothesized off-target effect of SRC TKIs on blinatumomab[21]

SRC TKIs antagonize blinatumomab through inhibition of LCK phosphorylation. We believe this off-target effect decreases cytotoxic immune cell activity promoted by blinatumomab and decreases long-term survival for adult ALL patients.

The second objective of this thesis is to explore the potential downstream signaling pathway that might rescue the antagonistic effect of the SRC TKIs. We investigate possible rescue pathways for off-target effects of TKIs on biTEs through the addition of interleukins (ILs). ILs are a type of cytokine that plays essential roles in activation and differentiation of immune cells, and the addition of ILs to cancer therapies have been a recent topic of interest in efforts to improve immune responses for greater tumor cell killing effects [24, 25, 26]. IL2, IL7, and IL15 belong to the common gamma chain family and activate three major signaling pathways that promote cellular survival and proliferation, including the JAK-STAT5 signaling pathway [27] (Figure 5). We hypothesized that the addition of ILs, including IL2 (T cell growth factor), IL7, and IL15 could rescue the off-target effects of SRC TKIs on blinatumomab through the activation of JAK-STAT5 signaling.

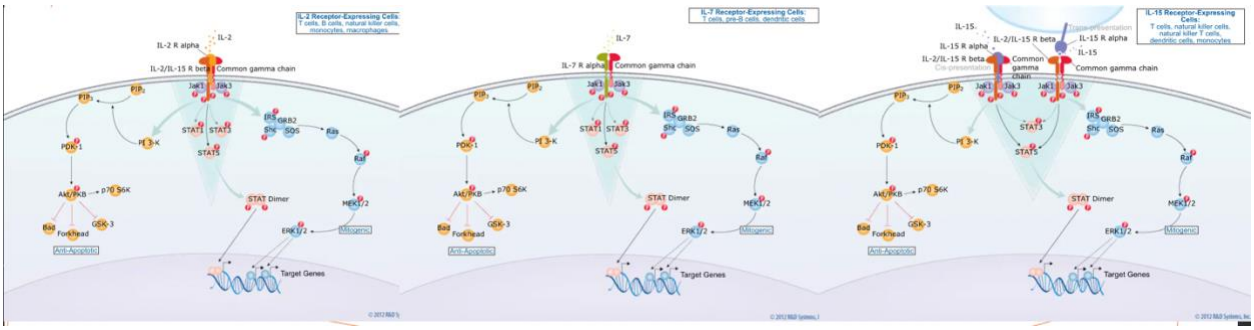


Figure 5. Three major signaling pathways involved in IL2 (left diagram), IL7 (middle diagram), and IL15 (right diagram)[27]

Common gamma ILs 2, 7, and 15 are activated by the JAK-STAT5 signaling pathway to promote immune cell activity. We hypothesize that the antagonistic off-target effects of SRC TKIs can be rescued through activation of the JAK-STAT5 signaling pathway to increase tumor cell killing effects in adult ALL patients.

With the conclusions of this study, we aim to explicate the significant role of TCR in combination therapy with biTEs and to report TKIs that will increase the efficacy of blinatumomab. Ultimately, we anticipate to propose potential therapeutic interactions that will improve ALL patient outcomes.

Chapter 2

Methods

Cell Culture

BV173 cells are a type of human Ph+ B cell precursor ALL that were used to show the effect of TKIs on LCK activity and CD19+ cell elimination. These cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 culture medium with Penicillin-Streptomycin-Glutamine (P/S/G; 100X) and 10% fetal bovine serum (FBS). All centrifugation steps with BV173 cells were performed at 500g for five minutes.

Jurkat cells are a type of human T lymphoblastoid cell line that were used as a system model to show the effect of TKIs on LCK activity and the effect of ILs on phosphorylated STAT5 (pSTAT5), which is essential for JAK-STAT5 signaling. Jurkat cells are CD3+ and have greater stability for preserving their viability and functionality in varying environments in comparison to peripheral blood mononuclear cell lines (PBMCs) and T cell lines. Additionally, Jurkat cells are more cost effective and thus were used during optimization trials of the experiment to model outcomes for replicates using PBMCs and T cell lines. The Jurkat cells were cultured in RPMI 1640 culture medium with P/S/G (100X) and 10% FBS. All centrifugation steps with Jurkats were performed at 500g for five minutes.

PBMCs and human T cells were cultured to also show the effect of TKIs on LCK activity and the effect of ILs on pSTAT5. PBMCs and human T cells are CD3+ that are found in adult ALL patients. Therefore, these cells reflect a more accurate immune cell response for adult ALL patients in comparison to Jurkat cells. However, the viability of these cells is sensitive to changing environmental conditions, and thus, these cells are difficult to culture and preserve.

PBMCs and human T cells were cultured separately in RPMI 1640 culture medium with P/S/G (100X) and 10% FBS. All centrifugation steps with PBMCs and T cells were performed at 250g for five minutes.

Flow Cytometry of pLCK Signaling

Flow cytometry is a useful laboratory practice for distinguishing cells by size, shape, granularity, and expression of extracellular surface and intracellular molecules [28]. In this experiment, a combination killing assay was performed and flow cytometry was used to distinguished between ALL cells (B cells) and T cells through intracellular staining and expression of CD3 and CD19 antigens. We measured i) T cell proliferation through LCK phosphorylation of CD3+ cells and ii) CD19+ cell elimination under various drug treatments using fluorescence-activated cell sorting (FACS).

Jurkat cells were serum starved overnight for approximately 16 hours prior to the experiment. Serial dilutions of the drug treatments were performed (Supplementary Table 1 in Appendix). BV173 cells were cocultured with serum starved Jurkat cells (2:1, BV173:Jurkat). Experimental samples were treated with diluted TKIs dasatinib and nilotinib in combination with 0.1ng blinatumomab (Supplementary Table 2 in Appendix). Control samples were prepared with serum-free media, and Jurkat cells were stimulated with CD4/CD28 ThermoFischer Dynabeads for the positive control (Supplementary Table 2 in Appendix). After two hours of incubation, the cells were collected, fixed with 4% PFA, and permeabilized with ice cold methanol (except for the unpermeabilized control) to allow for intracellular signaling analysis. The cells were placed on ice for 30 minutes, washed three times using 1% BSA in PBS, and stored in 4°C overnight.

The following day, the cells were blocked to prevent unspecific binding using FC block for 20 minutes at room temperature, and the experimental and positive control samples were stained for CD19+ (APC), CD3+ (PerCP-Cy5.5), and phosphorylated LCK (pLCK) Y394 (primary and secondary GFP). The samples were quantified using flow cytometry.

After the experiment was optimized using BV173 + Jurkat cell cocultures, a combination killing assay was prepared using BV173+T cell cocultures (2:1, BV173:T cells). Experimental samples were treated with diluted TKIs dasatinib, ponatinib, imatinib, and nilotinib in combination with 0.1ng blinatumomab (Supplementary Table 3 in Appendix). Control samples were prepared with serum-free media, and T cells were stimulated with CD4/CD28 ThermoFischer Dynabeads for the positive control (Supplementary Table 3 in Appendix). The samples were incubated for two hours, and identical steps proceeding incubation for the Jurkat+BV173 coculture replicates (described above) were followed.

IL-Rescue pLCK Signaling using Flow Cytometry

A combination drug treatment killing assay with BV173+PBMC cocultures were treated with ILs 2, 7, and 15, along with a combination of these ILs (marked as ILC), after treatment with TKI+blinatumomab therapies to test if ILs can rescue the antagonistic effects by increasing the proliferation of CD3+T cells and elimination of CD19+ cells.

The methodologies described above for the combination killing assay with BV173+T cell cocultures were followed for sample preparation and treatment with TKI+blinatumomab therapies (refer to Supplementary Table 4 in Appendix for list of samples). After the two-hour incubation step, ILs 2, 7, 15, and C were added to the experimental and control samples, and

samples were incubated for three days. After three days incubation, the cells were collected, fixed, and permeabilized to allow for intracellular signaling analysis. The cells were placed on ice for 30 minutes, washed three times using 1% BSA in PBS, and blocked to prevent unspecific binding using FC block for 20 minutes at room temperature. The experimental and positive control samples were stained for CD19+ (APC), CD3+ (PerCP-Cy5.5), and pLCK Y394 (primary and secondary GFP) and then were quantified using flow cytometry.

Drug Treatment Western Blots

Western blotting is a useful technique for separating and identifying specific proteins within various cell types. This technique accomplishes its task by utilizing three elements: separation by size, transfer to a solid support, and visually marking the target protein with the use of primary and secondary antibodies [29]. We performed a drug treatment western blot to evaluate LCK activation in Jurkat cells after four hours of treatment with SRC and non-SRC TKIs.

First, a drug treatment assay was prepared with approximately two million Jurkat cells in each sample. Jurkat cells were serum starved for two hours, and the control samples were prepped: the negative control consisted of unstimulated Jurkat cells, and the positive control consisted of Jurkat cells stimulated with CD3/CD28 magnetic beads. The samples were incubated for two more hours pretreatment. After four hours total of incubation, the experimental samples were treated with diluted TKIs dasatinib, ponatinib, imatinib, and nilotinib and were incubated for four hours. A cell lysis buffer consisting of 100uL lysis buffer, 900uL phosphate buffered saline (PBS), 4uL phenylmethylsulfonyl fluoride (PMSF), and 2uL PMSF protease inhibitor was prepared during treatment. Following treatment, the samples were centrifuged, the

supernatant was removed, and buffer was added to each cell pellet. The samples were placed in an ice bath for 30 minutes, vortexing every 10 minutes. After 30 minutes, the samples were centrifuged for 15 minutes, and the supernatant was removed.

Next, a Bicinchoninic acid (BCA) protein assay was performed to measure the pSTAT5 and pLCK concentrations in the cells. A Thermo Fisher BCA kit was used to prepare nine standards (Supplementary Table 5 in Appendix). Each standard and experimental sample was prepped by adding 5uL of the sample to 100uL of the working reagent (WR) in a 96 well plate. The plate was incubated at 37° C for 30 minutes, and then, all samples were cooled to room temperature. The samples were read using Envision manager software with a 562nm wave absorbance.

In preparation of the western blot, running and transfer buffers were made using 50mL of their respective liquids in addition to 950mL milli-Q water. Experimental samples were prepped with 10ug protein in 20uL sample buffer. A 2x lysis buffer was made with 500uL 1xPBS and 500uL NuPAGE LDS sample buffer and was added to the samples. The samples were placed in an 80°C bath for 10 minutes. The 4-12% NuPAGE gel was prepared, running buffer was added, and the wells were washed. The ladder was loaded with 15uL volume in each cell. The gel was run in 4°C with 150V, 3.00A, and 300W applied for two hours.

After two hours, the gel was transferred to a western blot transfer box. A 0.45 nitrocellulose membrane filter was used, and the box was tightly sealed to ensure proper transfer. The box was loaded into the device, and transfer buffer was added. The blot was run in 4°C with 150V, 3.00A, and 300W applied for two hours. Then, the membrane was carefully removed from the blot, and 5mL of 5% BSA in TBST was added to the membrane to block unspecific binding. The membrane was left covered in the shaker for one hour, and a primary antibody solution was

made using 5% BSA in TBST. After the one hour of blocking was complete, the membrane was stained with the primary antibody solution overnight in 4°C.

The next day, the membrane was washed with TBST for 15 minutes three times and then stained with a secondary antibody solution in room temperature for one hour. The membrane was washed with TBST for 15 minutes three times again. A chemiluminescent detection solution was made and added to the blot for one minute. The blot was imaged.

After imaging, the blot was placed in stripping buffer for 30 minutes, washed with dH2O for 15 minutes, and washed with TBST for 15 minutes. The blocking, staining, and imaging steps were repeated for all antibody conditions.

IL-Rescue Western Blots

Drug treatment western blot assays were treated with ILs 2, 7, 15, and C after treatment with TKIs to test if ILs can rescue the off-target effects that SRC TKIs have on biTEs through the activation of pSTAT5.

The methodologies described above for the drug treatment western blot were followed for sample preparation and treatment with TKIs. After treatment with TKIs, ILs 2, 7, 15, and C were added to the experimental and control samples (such as the samples listed in Supplementary Table 4 in the Appendix), and the samples were incubated for three days.

The cell lysis buffer was made after the three-day incubation period with ILs, and all methodologies proceeding treatment for the drug treatment western blots resumed as described above.

Analysis

FACS data from flow cytometry was analyzed using FCS express and FlowJo applications. Live cells were gated. Then, the live cell populations were gated for CD19+ (APC) and CD3+ (PerCP) cells. The cell counts for CD19+ cells are representative of live BV173 cells, and the cell counts for the CD3+ cells are representative of live Jurkat/T cells. Cell counts of pLCK+ cells (FITC) were measured in CD3+ cells and plotted into histograms. Individual and group sample data was computed for CD3+ and CD19+ cell counts, along with pLCK mean and standard deviation values.

Western blot images were analyzed using imageJ to compute the mean gray areas and backgrounds for the loading and experimental controls. The samples were standardized by subtracting the background areas from the sample readings. The standard curves were computed in Microsoft Excel and then were used to determine the protein concentrations. The pLCK and pSTAT5 values were normalized to the respective loading and negative controls and were plotted.

Chapter 3

Results

Flow Cytometry of pLCK Signaling

Table 1. Analyzed data from pLCK signaling experiment with BV173 + Jurkat cells.

	Cells/ Singlets CD3+ Mean FL1-A :: FITC-A	Cells/ Singlets CD3+ SD FL1-A :: FITC-A	Cells/ Singlets CD3+ Count	Cells/ Singlets CD19+ Count
A01 neg_jurkat_1.fcs	1414	1116	1654	52.0
A02 neg_jurk+BV173_1.fcs	1153	717	5157	1017
A03 neg_BV173_1.fcs	990	603	33082	5766
A04 unstained_1.fcs	1053	713	61034	9556
A05 unpermeabilized_1.fcs	15051	10848	41043	139712
A06 blincyto_1.fcs	32749	19195	2389	856
A07 dasatinib_1.fcs	16858	10706	24617	4122
A08 nilotinib_1.fcs	19227	13059	27472	5893
A09 positive_jurkat_1.fcs	33594	17381	358	745
B01 neg_jurkat_2.fcs	1193	821	1839	62.0
B02 neg_jurk+BV173_2.fcs	1139	695	71619	6861
B03 neg_BV173_2.fcs	998	628	62018	12286
B04 unstained_2.fcs	1082	722	70854	10524
B05 unpermeabilized_2.fcs	13728	10516	30955	183874
B06 blincyto_2.fcs	23449	14803	48190	19215
B07 dasatinib_2.fcs	16959	12217	23569	6201
B08 nilotinib_2.fcs	18695	12419	34304	8884
B09 positive_jurkat_2.fcs	21112	17827	129	172
Mean	*12247*	*8055*	*30016*	*23100*
SD	*11410*	*7118*	*25082*	*51277*

A combination drug treatment kill assay was performed with BV173+Jurkat cocultures. The cells were treated for 2 hours and stained with APC (CD19+), Per-CP (CD3+), and FITC (pLCK) antibodies. Results were insignificant and used for optimization of the experiment.

Combinations of dasatinib with blinatumomab and nilotinib with blinatumomab showed inconsistent, insignificant trends in CD3+ cell counts and CD19+ cell elimination.

Table 2. Analyzed data from pLCK signaling experiment with BV173 + T cells.

	Cells/ Singlets CD3+ FL1-A :: FITC-A	Cells/ Singlets CD3+ SD FL1-A :: FITC-A	Cells/ Singlets CD3+ Count	Cells/ Singlets CD19+ Count
A01 neg_TCell_1.fcs	147	161	559	0
A02 neg_BV_1.fcs	126	85.7	4786	0
A03 neg_TCell+BV_1.fcs	180	168	236	0
A04 unstained_1.fcs	161	145	586	0
A05 unperm_1.fcs	3718	4143	676	758
A06 ponatinib_1.fcs	1721	761	300	4.00
A07 imatinib_1.fcs	1877	1214	50.0	1.00
A08 dasatinib_1.fcs	1870	3085	133	0
A09 nilotinib_1.fcs	2224	1365	8320	0
A10 blincyto_1.fcs	1270	606	64.0	0
A11 pos_TCell_1.fcs	2617	1947	106	0
B01 neg_TCell_2.fcs	124	116	558	0
B02 neg_BV_2.fcs	153	304	268	0
B03 neg_TCell+BV_2.fcs	171	148	126	0
B04 unstained_2.fcs	156	156	505	1.00
B05 unperm_2.fcs	1293	1245	201	7477
B06 ponatinib_2.fcs	2375	5215	280	0
B07 imatinib_2.fcs	1570	712	25.0	0
B08 dasatinib_2.fcs	1484	773	164	0
B09 nilotinib_2.fcs	1424	1105	7557	3.00
B10 blincyto_2.fcs	1263	2639	186	0
B11 pos_TCell_2.fcs	3902	4184	43.0	0
Mean	*1356*	*1376*	*1170*	*375*
SD	*1150*	*1525*	*2403*	*1594*

A combination drug treatment kill assay was performed with BV173+T cell cocultures. The cells were treated for 2 hours and stained with APC (CD19+), Per-CP (CD3+), and FITC (pLCK) antibodies. Results were inconclusive due to low T cell count and staining error with CD19+ antibody. Results were used for optimization of experiment. Protocol was modified to increase cell counts, serum starve T cells for two hours prior to the experiment, stain cells with carboxyfluorescein succinimidyl ester (CFSE) prior to experiment, permeabilize the samples after the staining steps, and use CD19 (PE) antibody instead of CD19 (APC) during staining for future replicates.

Combinations of SRC TKIs (ponatinib/dasatinib) with blinatumomab and non-SRC TKIs (nilotinib/imatinib) with blinatumomab showed inconsistent, insignificant trends in CD3+ cell counts and CD19+ cell elimination.

Drug Treatment Western Blots

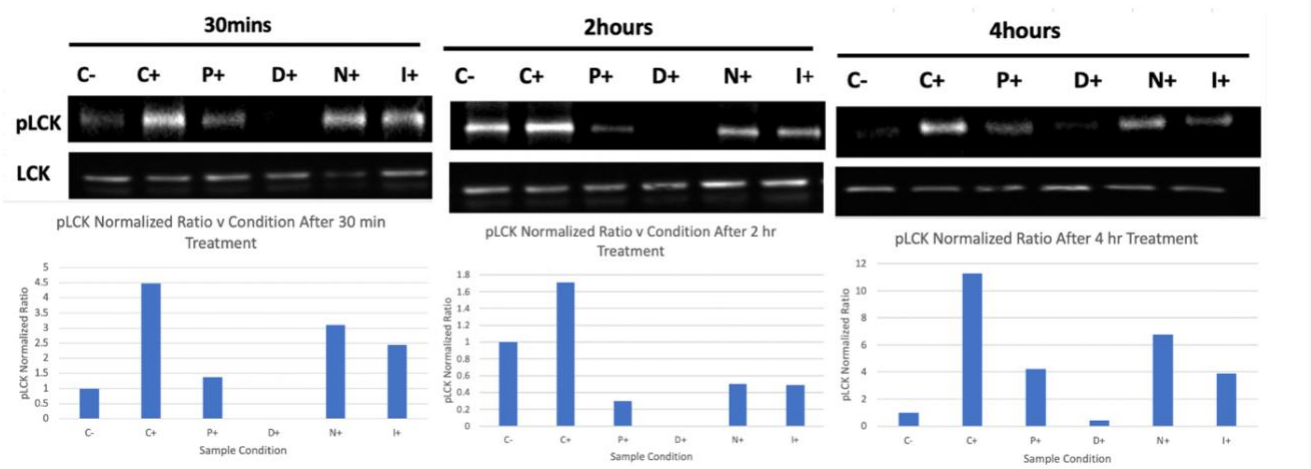


Figure 6. pLCK normalized ratio in Jurkat cells after 30 minutes, 2 hours, and 4 hours treatments with TKIs.

Jurkat cells were treated with TKIs for 30 minutes, 2 hours, and 4 hours, stained for LCK and pLCK (tyrosine 394), blotted, and imaged. Treatments with ponatinib and dasatinib showed pLCK inhibition on tyrosine 394. Refer to Supplementary Tables 6, 7, and 8 in Appendix for statistical analysis.

After 30 minutes, two hours, and four hours, samples treated with dasatinib showed pLCK inhibition on tyrosine 394. After 30 minutes and four hours, samples treated with ponatinib showed little LCK phosphorylation on tyrosine 394. Samples treated with non-SRC TKIs (nilotinib/imatinib) showed phosphorylation of LCK on tyrosine after 30 minutes and four hours.

Flow Cytometry of IL-Rescue Kill Assay

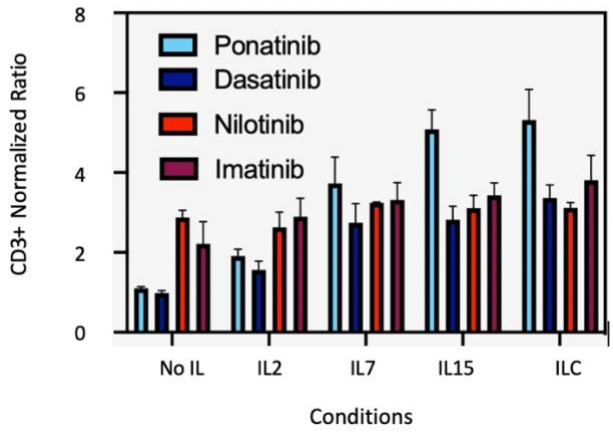


Figure 7. CD3+ normalized ratio in BV173 + PBMC cocultures treated with TKI + blinatumomab combination therapies and ILs 2, 7, 15, and C.

A combination drug treatment kill assay was performed with BV173+PBMC cocultures. The cells were treated for 2 hours with TKI+blinatumomab combinations, incubated with ILs for 3 days, and stained with Per-CP (CD3+) and FITC (pLCK) antibodies. Ponatinib/dasatinib+blinatumomab combinations showed rescue of CD3+ cells with the addition of ILs. Refer to Supplementary Table 4 in Appendix for statistical analysis.

Samples treated with the SRC TKIs ponatinib and dasatinib showed a significant increase in CD3+ cells with the addition of ILs. Samples treated with imatinib showed a slight increase with the addition of ILs. Samples treated with nilotinib did not show a significant increase in CD3+ cells with the addition of ILs.

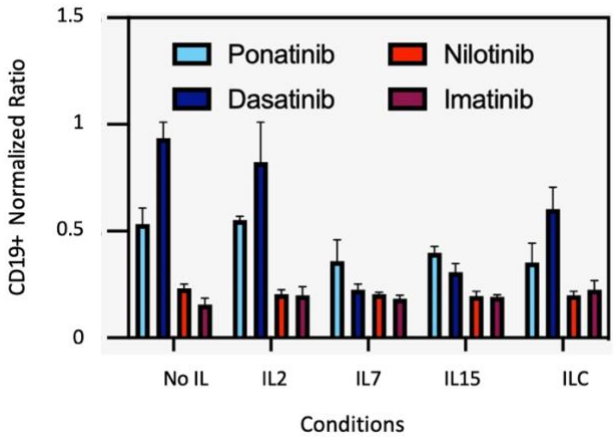


Figure 8. Normalized ratio of CD19+ cells in BV173 + PBMC cocultures treated with TKI + blinatumomab combination therapies and ILs 2, 7, 15, and C.

A combination drug treatment kill assay was performed with BV173+PBMC cocultures. The cells were treated for 2 hours with TKI+blinatumomab combinations, incubated with ILs for 3 days, and stained with CD19+ (APC) antibodies. Ponatinib/dasatinib+blinatumomab combinations showed greater CD19+ cell elimination with the addition of ILs. Refer to Supplementary Table 4 in Appendix for statistical analysis.

Samples treated with the SRC TKIs ponatinib and dasatinib showed a significant decrease in CD19+ cells with the addition of ILs 7, 15, and C. The addition of ILs 2, 7, 15, and C to samples treated with the non-SRC TKIs nilotinib and imatinib showed little to no difference in CD19+ cells in comparison to samples without added ILs.

IL-Rescue Western Blots

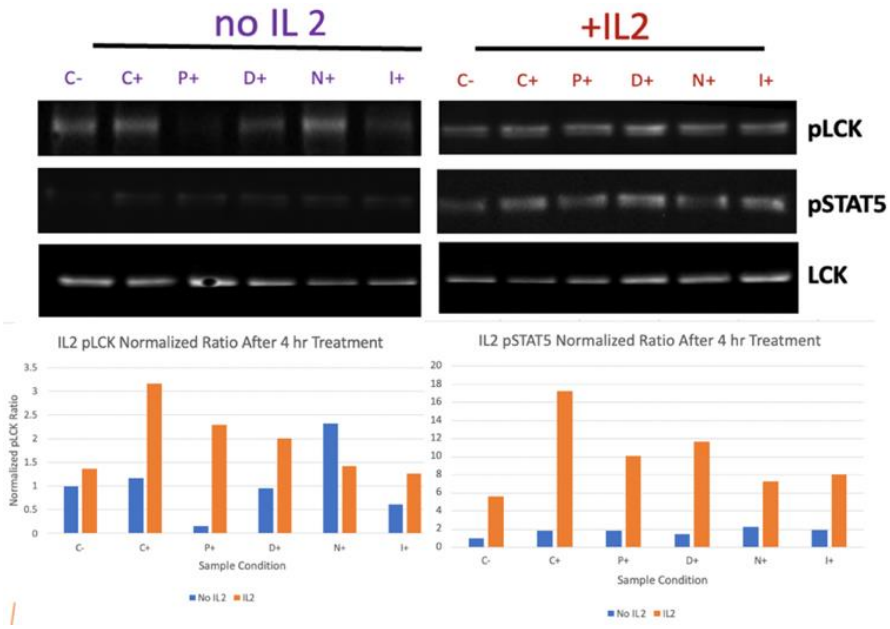


Figure 9. pLCK and pSTAT5 normalized ratios in IL-rescue western blot with IL2 after four-hour treatment.

A drug treatment killing assay was performed with Jurkat cells, and IL2 was added to the cells for 3 days. The samples were blotted and imaged. Ponatinib and dasatinib treatments show rescue of pLCK and pSTAT5 with the addition of IL2. Refer to Supplementary Table 9 in Appendix for statistical analysis.

Samples treated with ponatinib, dasatinib, and imatinib with the addition of IL2 showed greater LCK phosphorylation normalized ratios in comparison to samples under the same treatments with the addition of IL2. Samples treated with ponatinib, dasatinib, nilotinib, and imatinib with the addition of IL2 showed greater STAT5 phosphorylation normalized ratios in comparison to samples without IL2.

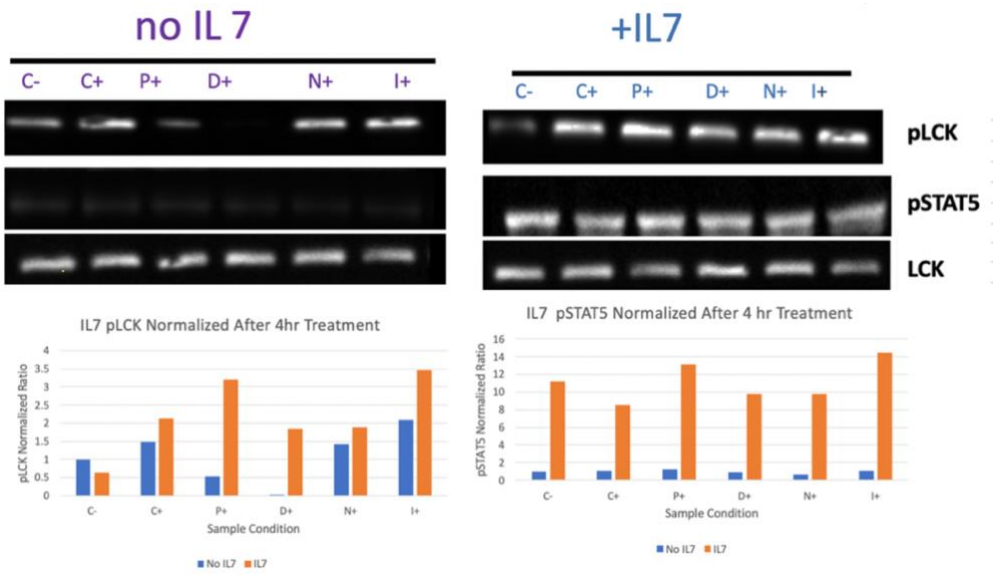


Figure 10. pLCK and pSTAT5 normalized ratios in IL-rescue western blot with IL7 after four-hour treatment.

A drug treatment killing assay was performed with Jurkat cells, and IL7 was added to the cells for 3 days. The samples were blotted and imaged. Ponatinib and dasatinib treatments show rescue of pLCK and pSTAT5 with the addition of IL7. Refer to Supplementary Table 10 in Appendix for statistical analysis.

Samples treated with ponatinib, dasatinib, nilotinib, and imatinib with the addition of IL7 showed greater LCK phosphorylation normalized ratios in comparison to samples under the same treatments without the addition of IL7. Samples treated with ponatinib, dasatinib, nilotinib, and imatinib with the addition of IL7 showed greater STAT5 phosphorylation normalized ratios in comparison to samples without IL7.

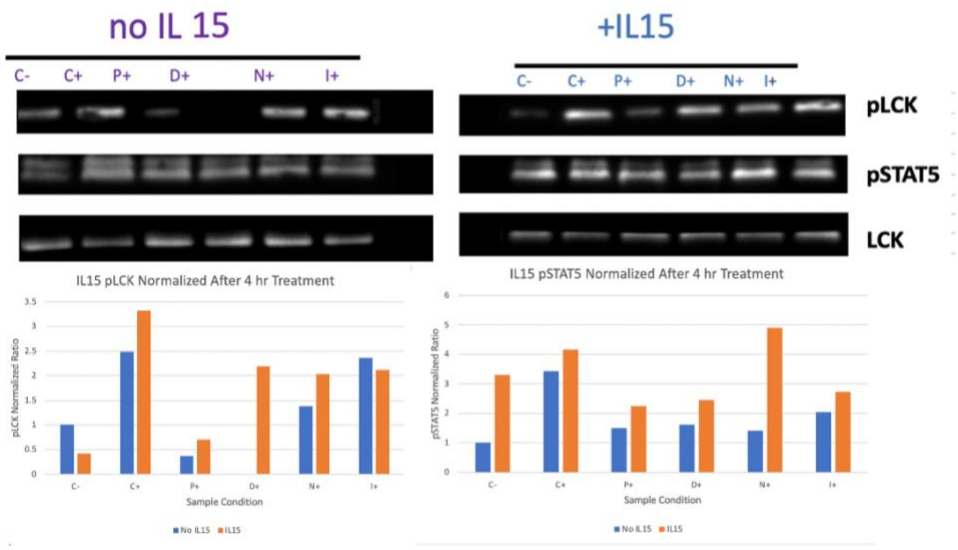


Figure 11. pLCK and pSTAT5 normalized ratios in IL-rescue western blot with IL15 after four-hour treatment.

A drug treatment killing assay was performed with Jurkat cells, and IL15 was added to the cells for 3 days. The samples were blotted and imaged. Ponatinib and dasatinib treatments show rescue of pLCK and pSTAT5 with the addition of IL15. Refer to Supplementary Table 11 in Appendix for statistical analysis.

Samples treated with ponatinib, dasatinib, nilotinib, and imatinib with the addition of IL15 showed greater LCK phosphorylation normalized ratios in comparison to samples under the same treatments without the addition of IL15. Samples treated with ponatinib, dasatinib, nilotinib, and imatinib with the addition of IL15 showed greater STAT5 phosphorylation normalized ratios in comparison to samples without IL15.

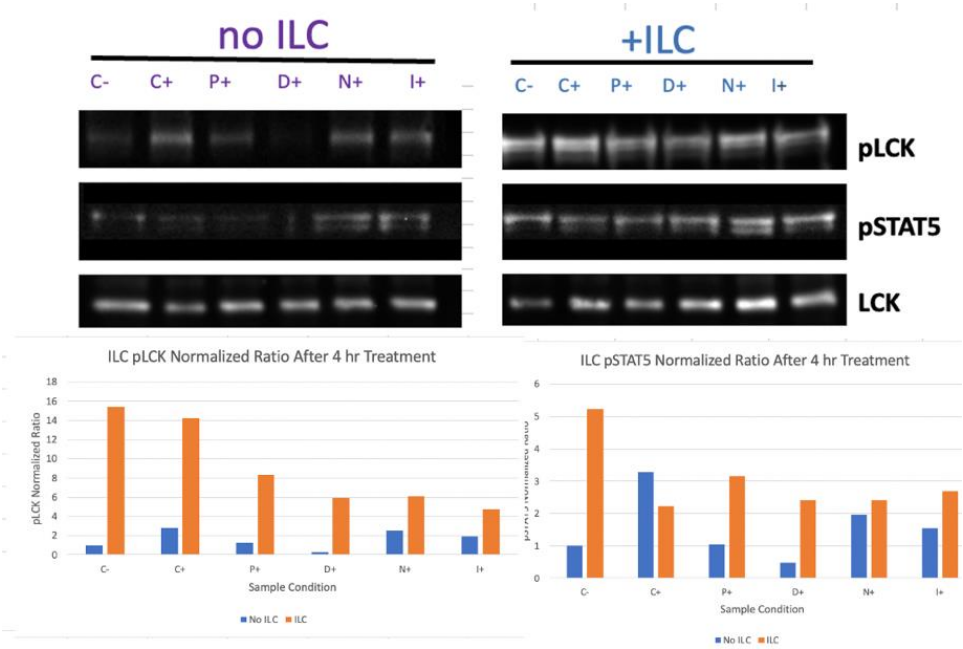


Figure 12. pLCK and pSTAT5 normalized ratios in IL-rescue western blot with ILC after four-hour treatment. A

A drug treatment killing assay was performed with Jurkat cells, and ILC was added to the cells for 3 days. The samples were blotted and imaged. Ponatinib and dasatinib treatments show rescue of pLCK and pSTAT5 with the addition of ILC. Refer to Supplementary Table 12 in Appendix for statistical analysis.

Samples treated with ponatinib, dasatinib, nilotinib, and imatinib with the addition of ILC showed greater LCK phosphorylation normalized ratios in comparison to samples under the same treatments without the addition of ILC. Samples treated with ponatinib, dasatinib, nilotinib, and imatinib with the addition of ILC showed greater STAT5 phosphorylation normalized ratios in comparison to samples without ILC.

Chapter 4

Discussion

Initial pLCK signaling flow cytometry results showed inconsistent, insignificant trends for BV173+Jurkat cells and BV173+T cell cocultures (Tables 1 & 2). These results were used to optimize the protocol by increasing cell counts, serum starving the T cells for two hours prior to the experiment, staining cells with carboxyfluorescein succinimidyl ester (CFSE) prior to the experiment, permeabilizing the samples after the staining steps, and using CD19 (PE) antibody instead of CD19 (APC) during staining for future replicates.

After optimization, the pLCK signaling flow cytometry results showed that samples treated with the SRC TKIs dasatinib/ponatinib did not have pLCK+ cells (Supplementary Figure 1 in Appendix), indicating that combination therapies of SRC TKIs with blinatumomab inhibit cytotoxic T cell activity. Blinatumomab as a single agent initiates an immune response to increase the tumor killing effect. Therefore, the absence of pLCK+ cells in the dasatinib/ponatinib+blinatumomab conditions validate previous findings that SRC TKIs antagonize biTEs through the inhibition of pLCK in immune cells [22].

Additionally, the pLCK signaling flow cytometry results showed the presence of pLCK+ cells in the samples treated with non-SRC TKIs nilotinib/imatinib with blinatumomab (Supplementary Figure 1 in Appendix). The presence of pLCK+ cells in these samples indicates that non-SRC TKIs in combination with blinatumomab initiates an immune response to increase the tumor cell killing effect. Thus, these results support the hypothesis that combinations of non-SRC TKIs have synergistic effects on biTEs.

The drug treatment western blots (Figure 6) and the IL-rescue western blots (Figures 9, 10, 11, & 12) showed that phosphorylation of LCK on tyrosine 394 is inhibited by SRC TKIs

and thus support the findings of the pLCK signaling flow cytometry results (Supplementary Figure 1 in Appendix). Notably, the drug treatment western blots showed the greatest difference in the pLCK normalized ratios between conditions after four hours of treatment (Figure 6); as a result, the treatment period for western blotting was optimized to four hours. Treatment with ponatinib showed a greater pLCK normalized ratio in comparison to treatment with dasatinib (Figure 6), suggesting that ponatinib has a less inhibitory effect on the phosphorylation of LCK on tyrosine 394 than dasatinib. Treatment with nilotinib showed a greater pLCK normalized ratio in comparison to treatment with imatinib across all three time points (Figure 6), which suggests that nilotinib has less inhibitory effects on the phosphorylation of LCK and promotes more cytotoxic T cell activation in comparison to imatinib.

While treatment with SRC TKIs showed inhibition of pLCK signaling based on the drug treatment kill assay results (Supplementary Figure 1 in Appendix), the IL-rescue kill assay results showed rescue of T cell activation in treatments with SRC TKIs with the addition of ILs 2, 7, 15, and C (Figure 7). Treatment with ponatinib showed the greatest increase in CD3+ cells with the addition of ILs 2, 7, 15, and C in comparison to the other TKI treatments (Figure 7). Addition of IL7 to the ponatinib condition showed approximately a 2X increase in CD3+ cells from the condition without added ILs, while addition of ILs 15 and C showed approximately a 3X increase in CD3+ cells (Figure 7). This suggests that the addition of ILs 15 and C will have the greatest rescue effects for ponatinib treatment.

The IL-rescue western blots showed that the addition of ILC had the greatest effect on increasing the phosphorylation of LCK in ponatinib treatments in comparison to the addition of ILs 2, 7, and 15 (Figures 9, 10, 11, & 12; Supplementary Tables 9, 10, 11, & 12 in Appendix). However, the addition of ILC to ponatinib treatments did not have a significant effect on

increasing phosphorylation of STAT5 (Figures 9, 10, 11, & 12; Supplementary Tables 9, 10, 11, & 12 in Appendix). Therefore, the addition of ILC showed greater rescue of the antagonistic effects of ponatinib through the pLCK signaling pathway than through the pSTAT5 signaling pathway.

Although the IL-rescue kill assay flow cytometry results showed the greatest rescue effects with the addition of IL15 to ponatinib treatments (Figure 7), the IL-rescue western blots showed that the addition of IL15 to ponatinib treatments had the least effects on increasing the phosphorylation of LCK and STAT5 in comparison to the addition of ILs 2, 7, and C (Figures 9, 10, 11, & 12; Supplementary Tables 9, 10, 11, & 12 in Appendix). This suggests that the addition of IL15 to ponatinib treatments rescues the immune response through a combination of signaling pathways, including potential pathways that have not yet been investigated.

In addition, the IL-rescue western blots showed that the addition of IL7 to ponatinib treatments had the greatest effect on increasing the phosphorylation of STAT5, followed by the addition on IL2 (Figures 9, 10, 11, & 12; Supplementary Tables 9, 10, 11, & 12 in Appendix). Although the addition of ILs C and 15 to ponatinib treatments showed little increase in the phosphorylation of pSTAT5 from the ponatinib samples without added ILs, the phosphorylation of STAT5 increased in all the ponatinib treatments with added ILs (Figures 9, 10, 11, & 12; Supplementary Tables 9, 10, 11, & 12 in Appendix).

The IL-rescue kill assay flow cytometry results also showed that the addition of ILs 2, 7, 15, and C increased CD3+ cells in dasatinib treatments (Figure 7). The addition of IL2 to dasatinib treatment showed little increase in CD3+ cells in comparison to dasatinib treatment without added ILs. The addition of ILs 7, 15, and C showed approximately a 2X increase in CD3+ cells from the dasatinib treatment without added ILs. These results indicate that ILs 7, 15

and C will be equally effective and more effecting that IL2 for rescuing the off-target effects of dasatinib.

The IL-rescue western blots with dasatinib treatment showed that the addition of ILs 7, 15, and C had differing effects on rescuing the pLCK and pSTAT5 pathways (Figures 9, 10, 11, & 12; Supplementary Tables 9, 10, 11, & 12 in Appendix). The addition of ILC to dasatinib treatment had the greatest effect on increasing the phosphorylation of LCK in comparison to the addition of ILs 2, 7, and 15. This result is similar to the IL-rescue western blot results with ponatinib treatment and thus suggests that the addition of ILC has the greatest rescuing effect on the phosphorylation of LCK in SRC TKIs treatments. The addition of IL15 to dasatinib treatment had the next greatest effect on the phosphorylation of LCK, followed by the addition of ILs 7 and 2 respectively. The trends found with the addition of ILs to dasatinib treatment reflected the trends found for the addition of ILs to ponatinib treatment (Figures 9, 10, 11, & 12; Supplementary Tables 9, 10, 11, & 12 in Appendix). The phosphorylation of STAT5 increased in all the dasatinib treatments with added ILs, with the addition of ILs 2 and 7 to dasatinib treatments having the greatest effects on the phosphorylation of STAT5, followed by ILs C and 15 respectively.

Notably, the flow cytometry results of the IL-rescue drug treatment assay show that the addition of ILs could enhance the T cell response with imatinib treatment; however, the potential adverse effects of added ILs, such as cytokine release syndrome and tumor cell growth, are important to consider and should be further investigated before being used in treatment. Added ILs with nilotinib treatment did not show effects on T cell activity in the IL-rescue flow cytometry results. The IL-rescue western blot results showed that the addition of ILs 2, 7, 15, and

C increased pSTAT5 activation in nilotinib and imatinib treatments in addition to the SRC TKI treatments.

The results of the CD19+ cell elimination showed that the addition of ILs to SRC TKI treatments significantly increased the tumor cell killing effect (Figure 8). This supports the IL-rescue western blot showing the increased pLCK and pSTAT5 signaling pathways with the addition of ILs to SRC TKI treatments (Figures 9, 10, 11, & 12; Supplementary Tables 9, 10, 11, & 12 in Appendix), as the increased immune responses led to a greater tumor cell killing effect. Although the IL-rescue western blots showed increased pSTAT5 activation in non-SRC TKI treatments, nilotinib and imatinib did not have significant CD19+ cell elimination with the addition of ILs (Figure 8).

Overall, the results of the IL-rescue drug treatment assays show that the addition of ILs rescue off-target effects of SRC TKIs on blinatumomab through the activation of the JAK-STAT5 pathway. These findings explicate the ongoing clinical findings that the combination of dasatinib and blinatumomab have shown promising results, despite previous *in vitro* studies showing the antagonistic effects of SRC TKIs on biTEs [22]. SRC TKIs antagonize biTEs through inhibition of the pLCK signaling pathway. With the addition of ILs, the efficacy of SRC TKI+biTE combination therapies can be rescued through activation of the JAK-STAT5 pathway. These findings provide hope for improving treatments and patient outcomes for adult ALL.

Limitations of this study

Jurkat cells were used in the flow cytometry experiments, as well as the IL-rescue western blot experiment. Although Jurkat cells do not reflect the immune responses in ALL patients as

accurately as PBMCs and human T cells, these cells were used to optimize the protocols due to their stability for preserving their viability and functionality in varying environment. Additional flow cytometry of pLCK signaling replicates with PBMCs (Supplementary Figure 2 in Appendix) were performed; however, due to human error and fragile viability of PBMCs, these replicates were used to optimize the protocols. Future studies should further investigate the findings of this study in BV173+PBMC/T cell cocultures to better predict the outcomes of ALL patients treated with combinational therapies of TKIs and biTEs.

In addition, the drug treatment western blot after the two-hour treatment greatly deviated from the trends in the western blots after 30 minutes and four-hours of treatment, and these differences were concluded to be due to error in the transferring step of the protocol. Future studies should perform additional replicates to produce results of higher statistical power at this time point. Similarly, future studies should study the results of the IL-rescue western blots across multiple time points to better understand the trends of these treatments overtime. Multiple time points during the IL-rescue western blot experiments were not obtained in this study due to time limitations.

Future directions

Future studies can further investigate the conclusions of this study to improve treatment and patient outcomes for adults with Ph+ ALL.

IL-rescue flow cytometry results showed that treatments with ponatinib and dasatinib with the addition of ILs 2, 7, 15, and C had the greatest CD19+ cell elimination and thus the greatest tumor cell killing effect. These results suggest that ponatinib and dasatinib treatments

with additional IL therapeutics should be clinically tested as a frontline treatment for adults with Ph+ ALL. Dasatinib as a single agent has shown promising results as a frontline treatment for adult Ph+ ALL [30], but combinational therapies of SRC TKIs+biTE with the addition of ILs have yet to be clinically investigated.

Moreover, the IL-rescue flow cytometry and drug treatment western blot results suggest that nilotinib treatment has greater pLCK signaling and tumor cell killing effects in comparison to imatinib. Future clinical studies should investigate the efficacies of TKIs of the same family to one another in combinational treatments to optimize non-SRC TKIs+blinatumomab therapies.

While the results of the IL-rescue flow cytometry and western blot drug treatment assays show that addition of ILs to SRC TKIs+blinatumomab have promising effects for immune response and tumor cell killing, previous studies have found that ILs also play a role in tumor growth and metastasis [25, 26, 31]. The combination of ILs 2, 7, and 15 (ILC) tested in this study showed the greatest effects on rescuing pLCK signaling in SRC TKIs+blinatumomab treatments, yet this combination of ILs has not yet been tested in clinical trials. Notably, although IL7 has been found to play a role in T cell development and survival [32], this IL is not naturally produced in the body and thus would need to be an added therapeutic in the treatments. Additionally, IL7 can promote the growth of T cell ALL and thus should not be used in treatments for patients with T cell precursor ALL [32]. The effects of using these ILs as added therapeutics should be studied in future clinical trials to better understand how they can be used to increase the efficacies of SRC TKIs+biTE combinational therapies.

ILs 4, 9, and 21 belong to the common gamma chain family, along with the ILs tested in this study (2, 7, and 15). ILs 4 and 9 have been found to promote cancer growth, while IL21 is antitumoral. Additionally, previous studies show that IL1 can have antitumoral immunity and

may block metastasis. ILs 18 and 36 are in the IL-1 superfamily and have also shown antitumoral effects. [24] Therefore, future studies should study the potential rescue effects of ILs 1, 18, 21, and 36 to SRC TKI+blinatumomab treatments for adult Ph+ ALL.

Although the IL-rescue kill assay flow cytometry results showed the addition of IL15 significantly increased the amount of CD3+ cells in ponatinib treatments, the IL-rescue western blot results showed the addition of IL15 did not have a significant effect on pLCK or pSTAT5 pathways. Future studies should investigate additional signaling pathways that IL15 impacts in order to rescue the antagonistic effects of SRC TKIs.

Lastly, future studies should further investigate the efficacies of combinational therapies with other TKIs, such as masitinib, with blinatumomab. Masitinib is currently being studied in clinical trials to treat various cancers, including acute myeloid leukemia, and similar to ponatinib and dasatinib, masitinib targets LCK and LCK-like proteins [35]. Based on the results of the IL-rescue western blots, which showed the addition of ILC had the greatest effects on the phosphorylation of LCK in ponatinib and dasatinib treatments, future studies should investigate the effects of ILC in treatments with additional SRC TKIs, such as masitinib. These conclusions will ultimately help researchers and medical professionals report TKIs that will increase the efficacy of blinatumomab and propose potential therapeutic interactions that will improve ALL patient outcomes.

Appendix

Supplementary Tables and Figures

Supplementary Table 1. Drug concentrations used in all drug treatment assays.

Drug	Stock	Serial Dilution			Working Concentration
Ponatinib	10mM	10uL in 900uL	100uM	10uL in 990uL	**1uM**
Dasatinib	10mM	10uL in 990uL	100uM	10uL in 990uL	**1uM**
Nilotinib	10mM	10uL in 990uL	100uM	10uL in 990uL	**1uM**
Imatinib	10mM	10uL in 990uL	100uM	10uL in 990uL	**1uM**
Blinatumomab	12.5ug/mL	8uL in 992uL	100ng/mL	10uL in 990uL	**1ng/mL**

Supplementary Table 2. Samples for pLCK signaling experiment with BV173 + Jurkat cells.

Condition	Jurkats	BV173s	Blinatumomab (0.1ng/ml)	Nilotinib (130nM)	Dasatinib (10nM)	CD4/CD28 Beads
Positive Blinatumomab Control	Y	Y	Y	N	N	N
Experimental 1 Blina.+Dasatinib	Y	Y	Y	N	Y	N
Experimental 2 Blina.+Nilotinib	Y	Y	Y	Y	N	N
Negative Jurkat Control	Y	N	N	N	N	N
Negative BV173 Control	N	Y	N	N	N	N
Negative Jurkat+BV173 Control (also labeled Unstained Control)	Y	Y	N	N	N	N
Positive Jurkat Control	Y	N	N	N	N	Y
Unpermeabilized Control	Y	Y	N	N	N	N

Supplementary Table 3. Samples for pLCK signaling experiment with BV173 + T cells.

Condition	T Cells	BV173s	Blinatumomab (0.1ng/ml)	Ponatinib (10nM)	Imatinib (130nM)	Dasatinib (10nM)	Nilotinib (130nM)	CD4/CD28 Beads
Positive Blinatumomab Control	Y	Y	Y	N	N	N	N	N
Experimental 1 Blina.+Ponatinib	Y	Y	Y	Y	N	N	N	N
Experimental 2 Blina.+Imatinib	Y	Y	Y	N	Y	N	N	N
Experimental 3: Blina.+Dasatinib	Y	Y	Y	N	N	Y	N	N
Experimental 4: Blina.+Nilotinib	Y	Y	Y	N	N	N	Y	N
Negative T Cell Control	Y	N	N	N	N	N	N	N
Negative BV173 Control	N	Y	N	N	N	N	N	N
Negative T Cell+BV173 Control (also labeled as Unstained Control)	Y	Y	N	N	N	N	N	N
Positive T Cell Control	Y	N	N	N	N	N	N	Y
Unpermeabilized Control	Y	Y	N	N	N	N	N	N

Supplementary Table 4. Samples and analyzed flow cytometry data (mean values) for IL-rescue kill assay.

Sample	ALL Cell Growth	T Cell Expansion	CD4+	CD8+
Negative BV173 Control	18636.2104	6.26239489	3.259331698	2.794293945
BV173+Dasatinib	1198.053863	46.04443527	29.04615338	13.56747676
BV173+Imatinib	18051.30953	10.25094485	6.56266294	3.352423941
BV173+Nilotinib	4153.572346	13.09686469	7.646385684	3.600780398
BV173+Ponatinib	2482.273539	26.55546445	15.5603356	10.81393451
BV173+PBMC	55.45525545	10016.94996	4322.853639	5375.87827
PBMC+Blinatumomab	14.86202143	4937.128987	2374.336866	2385.208628
PBMC+CD4/CD28 beads	68.17759379	6989.726528	3843.99451	2809.006895
BV173+PMBC+ILC	69.94250306	12747.78568	6825.452303	4681.807447
BV173+PBMC+Dasatinib+Blinatumomab	264.6329244	7923.808377	4447.537711	3224.168238
BV173+PBMC+Dasatinib+Blinatumomab+IL15	464.0977201	11436.57001	6851.573504	4248.164643
BV173+PBMC+Dasatinib+Blinatumomab+IL2	508.3547678	12550.22979	7017.586175	5073.708307
BV173+PBMC+Dasatinib+Blinatumomab+IL7	193.0889862	6914.847834	4154.057466	2618.260953
BV173+PBMC+Dasatinib+Blinatumomab+ILC	205.8802037	6199.411376	3739.220245	2336.365191
BV173+PBMC+Blinatumomab	512.6210795	12808.66632	7435.46489	5182.814011
BV173+PBMC+Blinatumomab+ILC	308.3163165	9534.542747	5551.593727	3865.678055
Negative BV173+PBMC Control	8234.907155	3895.33424	2129.92811	1668.311856
BV173+PBMC+Imatinib+Blinatumomab	430.5315856	12644.09699	7117.231793	5367.112981
BV173+PBMC+Imatinib+Blinatumomab+IL15	492.766073	14120.14094	8100.442396	5845.316329
BV173+PBMC+Imatinib+Blinatumomab+IL2	490.9164977	13584.62726	7604.730577	5779.626525
BV173+PBMC+Imatinib+Blinatumomab+IL7	404.4953022	12240.68268	6879.767478	5188.490691
BV173+PBMC+Imatinib+Blinatumomab+ILC	356.1193687	11389.30984	6485.943763	4769.719848
BV173+PBMC+Nilotinib+Blinatumomab	523.9408356	14742.575	8302.363437	6257.230989
BV173+PBMC+Nilotinib+Blinatumomab+IL15	492.1615983	14021.03123	7969.314484	5875.803551
BV173+PBMC+Nilotinib+Blinatumomab+IL2	475.2766633	13483.88054	7649.233505	5655.808394
BV173+PBMC+Nilotinib+Blinatumomab+IL7	413.1480507	13263.71472	7472.48673	5624.289199
BV173+PBMC+Nilotinib+Blinatumomab+ILC	310.8433305	10967.91775	6298.367382	4542.152406
BV173+PBMC+Ponatinib+Blinatumomab	566.7418071	15058.52174	8759.411265	5923.519875
BV173+PBMC+Ponatinib+Blinatumomab+IL15	619.6919659	14827.65362	9022.447151	5415.718736
BV173+PBMC+Ponatinib+Blinatumomab+IL2	506.8937033	13925.40342	8123.03082	5461.814807
BV173+PBMC+Ponatinib+Blinatumomab+IL7	193.9061898	8314.442869	5065.191526	3073.674989
BV173+PBMC+Ponatinib+Blinatumomab+ILC	251.886988	8614.034601	5208.101014	3190.457028

A combination drug treatment kill assay was performed with BV173+PBMC cocultures. The cells were treated for 2 hours with TKI+blinatumomab combinations, incubated with ILs for 3 days, and stained with APC (CD19+), Per-CP (CD3+), and FITC (pLCK) antibodies. CD19+ was a measure of ALL Cell Growth. CD3+ was a measure of T cell expansion. Ponatinib/dasatinib+blinatumomab combinations showed rescue of CD3+ cells with the addition of ILs.

Supplementary Table 5. Standards for drug treatment western blot.

Standard ID	Condition
A	300uL stock
B	375uL stock + 125uL PBS
C	325uL stock + 325uL PBS
D	175uL vial B + 175uL PBS
E	325uL vial C + 325uL PBS
F	325uL vial E + 325uL PBS
G	325uL vial F + 325uL PBS
H	100uL vial G + 400uL PBS
I	100uL PBS

Key for Samples in Tables 6, 7, 8, 9, 10, 11, and 12

Sample	Condition
C-	unstimulated Jurkats
C+	CD4/CD28 stimulated Jurkats
P+	Jurkats treated with ponatinib
D+	Jurkats treated with dasatinib
N+	Jurkats treated with nilotinib
I+	Jurkats treated with imatinib

Supplementary Table 6. Analyzed data for drug treatment western blot at 30 minutes.

Sample	Mean Gray	Mean Gray Background	Loading Mean Gray	Loading Mean Gray Background	Inverted Mean Gray	Inverted Mean Gray Background	Inverted Loading Mean Gray	Inverted Loading Mean Gray Background	Net Protein	Net Control	Ratio	Normalized Ratio
C-	53.615	0.775	85.847	5.378	201.385	254.225	169.153	249.622	52.84	80.469	0.656650387	1
C+	191.095	8.653	68.582	6.567	63.905	246.347	186.418	248.433	182.442	62.015	2.941901153	4.480163586
P+	78.93	3.467	91.487	7.659	176.07	251.533	163.513	247.341	75.463	83.828	0.90021234	1.370915722
D+	0.673	0.022	74.781	7.234	254.327	254.978	180.219	247.766	0.651	67.547	0.009637734	0.014677116
N+	154.071	1.526	80.684	5.739	100.929	253.474	174.316	249.261	152.545	74.945	2.035425979	3.099710316
I+	145.964	5.859	92.964	5.91	109.036	249.141	162.036	249.09	140.105	87.054	1.609403359	2.450928821

Jurkat cells were treated with TKIs for 30 minutes stained for LCK and pLCK (tyrosine 394), blotted, and imaged. Treatments with ponatinib and dasatinib showed pLCK inhibition on tyrosine 394. Refer to Key (above) for sample conditions.

Supplementary Table 7. Analyzed data for drug treatment western blot at two hours.

Sample	Mean Gray	Mean Gray Background	Loading Mean Gray	Loading Mean Gray Background	Inverted Mean Gray	Inverted Mean Gray Background	Inverted Loading Mean Gray	Inverted Loading Mean Gray Background	Net Protein	Net Control	Ratio	Normalized Ratio
C-	49.394	9.127	80.016	7.18	205.606	245.873	174.984	247.82	40.267	72.836	0.552844747	1
C+	124.145	6.321	67.745	8.694	130.855	248.679	187.255	246.306	117.824	59.051	1.995292205	3.609136589
P+	41.083	6.892	89.729	14.279	213.917	248.108	165.271	240.721	34.191	75.45	0.453161034	0.819689499
D+	0.487	0.48	75.634	10.302	254.513	254.52	179.366	244.698	0.007	65.332	0.000107145	0.000193807
N+	90.858	6.783	34.377	9.333	164.142	248.217	220.623	245.667	84.075	25.044	3.357091519	6.072394712
I+	92.374	7.684	88.074	12.914	162.626	247.316	166.926	242.086	84.69	75.16	1.126796168	2.038178302

Jurkat cells were treated with TKIs for 2 hours, stained for LCK and pLCK (tyrosine 394), blotted, and imaged. Results insignificant due to transferring error. Refer to Key (above Supplementary Table 6) for sample conditions.

Supplementary Table 8. Analyzed data for drug treatment western blot at four hours.

Sample	Mean Gray	Mean Gray Background	Loading Mean Gray	Loading Mean Gray Background	Inverted Mean Gray	Inverted Mean Gray Background	Inverted Loading Mean Gray	Inverted Loading Mean Gray Background	Net Protein	Net Control	Ratio	Normalized Ratio
C-	8.507	0.097	23.891	3.661	246.493	254.903	231.109	251.339	8.41	20.23	0.415719229	1
C+	95.782	2.654	23.385	3.53	159.218	252.346	231.615	251.47	93.128	19.855	4.690405439	11.28262807
P+	36.643	0.513	23.966	3.458	218.357	254.487	231.034	251.542	36.13	20.508	1.761751512	4.237839843
D+	6.727	0.053	40.275	3.621	248.273	254.947	214.725	251.379	6.674	36.654	0.182081083	0.437990523
N+	62.674	0.059	25.783	3.514	192.326	254.941	229.217	251.486	62.615	22.269	2.811756253	6.763594411
I+	31.358	0.053	23.12	3.808	223.642	254.947	231.88	251.192	31.305	19.312	1.621012842	3.89929724

Jurkat cells were treated with TKIs for 4 hours, stained for LCK and pLCK (tyrosine 394), blotted, and imaged. Treatments with ponatinib and dasatinib showed pLCK inhibition on tyrosine 394. Refer to Key (above Supplementary Table 6) for sample conditions.

Supplementary Table 9. Analyzed data for pLCK (Top) and pSTAT-5 (Bottom) in IL-rescue western blot with IL2 after four hours of treatment.

pLCK												
Sample	Mean Gray	Mean Gray Background	Loading Mean Gray	Loading Mean Gray Background	Inverted Mean Gray	Inverted Mean Gray Background	Inverted Loading Mean Gray	Inverted Loading Mean Gray Background	Net Protein	Net Control	Ratio	Normalized Ratio
no IL2												
C-	60.631	18.552	104.27	0.04	194.369	236.448	150.73	254.96	42.079	104.23	0.403712943	1
C+	56.869	18.183	81.944	0.063	198.131	236.817	173.056	254.937	38.686	81.881	0.47246614	1.170302188
P+	14.31	8.373	95.869	0.75	240.69	246.627	159.131	254.25	5.937	95.119	0.062416552	0.154606269
D+	41.373	14.254	72.385	2.274	213.627	240.746	182.615	252.726	27.119	70.111	0.38680093	0.958108817
N+	74.587	25.024	53.04	0.242	180.413	229.976	201.96	254.758	49.563	52.798	0.93872874	2.32523816
I+	34.627	20.167	58.579	0.611	220.373	234.833	196.421	254.389	14.46	57.968	0.249447971	0.617884504
IL2												
C-	59.715	17.278	77.444	0.444	195.285	237.722	177.556	254.556	42.437	77	0.55112987	1.36515284
C+	77.875	16.66	47.896	0.011	177.125	238.34	207.104	254.989	61.215	47.885	1.278375274	3.166545185
P+	80.446	14.946	70.473	0.007	174.554	240.054	184.527	254.993	65.5	70.466	0.929526296	2.302443639
D+	99.5	15.074	104.115	0.115	155.5	239.926	150.885	254.885	84.426	104	0.811788462	2.010806135
N+	80.541	28.473	90.791	0.095	174.459	226.527	164.209	254.905	52.068	90.696	0.574093676	1.422034359
I+	78.088	11.426	129.581	0.074	176.912	243.574	125.419	254.926	66.662	129.507	0.514736655	1.275006573

pSTAT-5												
Sample	Mean Gray	Mean Gray Background	Loading Mean Gray	Loading Mean Gray Background	Inverted Mean Gray	Inverted Mean Gray Background	Inverted Loading Mean Gray	Inverted Loading Mean Gray Background	Net Protein	Net Control	Ratio	Normalized Ratio
no IL2												
C-	19.632	12.667	104.27	0.04	235.368	242.333	150.73	254.96	6.965	104.23	0.066823371	1
C+	23.523	13.31	81.944	0.063	231.477	241.69	173.056	254.937	10.213	81.881	0.124729791	1.866559382
P+	24.43	12.659	95.869	0.75	230.57	242.341	159.131	254.25	11.771	95.119	0.12375025	1.851900721
D+	19.616	12.717	72.385	2.274	235.384	242.283	182.615	252.726	6.899	70.111	0.098401107	1.472555257
N+	23.318	12.562	73.04	0.242	231.682	242.438	181.96	254.758	10.756	72.798	0.147751312	2.211072395
I+	19.764	12.329	58.579	0.611	235.236	242.671	196.421	254.389	7.435	57.968	0.12826042	1.91939462
IL2												
C-	46.099	17.079	77.444	0.444	208.901	237.921	177.556	254.556	29.02	77	0.376883117	5.639989558
C+	73.546	18.342	47.896	0.011	181.454	236.658	207.104	254.989	55.204	47.885	1.152845359	17.25212803
P+	65.224	17.526	70.473	0.007	189.776	237.474	184.527	254.993	47.698	70.466	0.676893821	10.12959698
D+	98.947	17.592	104.115	0.115	156.053	237.408	150.885	254.885	81.355	104	0.782259615	11.70637756
N+	62.783	18.645	90.791	0.095	192.217	236.355	164.209	254.905	44.138	90.696	0.486658728	7.282762272
I+	86.803	17.401	129.581	0.074	168.197	237.599	125.419	254.926	69.402	129.507	0.535893813	8.019556654

A drug treatment killing assay was performed with Jurkat cells, and IL2 was added to the cells for 3 days. The samples were blotted and imaged. Ponatinib and dasatinib treatments show rescue of pLCK and pSTAT5 with the addition of IL2. Refer to Key (above Supplementary Table 6) for sample conditions.

Supplementary Table 10. Analyzed data for pLCK (Top) and pSTAT-5 (Bottom) in IL-rescue western blot with IL7 after four hours of treatment.

pLCK												
Sample	Mean Gray	Mean Gray Background	Loading Mean Gray	Loading Mean Gray Background	Inverted Mean Gray	Inverted Mean Gray Background	Inverted Loading Mean Gray	Inverted Loading Mean Gray Background	Net Protein	Net Control	Ratio	Normalized Ratio
no IL7												
C-	64.967	2.64	118.092	9.746	190.033	252.36	136.908	245.254	62.327	108.346	0.57525889	1
C+	87.888	2.464	110.358	9.498	167.112	252.536	144.642	245.502	85.424	100.86	0.84695618	1.472304362
P+	29.819	2.088	98.925	8.718	225.181	252.912	156.075	246.282	27.731	90.207	0.30741517	0.534394463
D+	3.491	1.578	118.494	9.451	251.509	253.422	136.506	245.549	1.913	109.043	0.01754354	0.03049677
N+	92.422	2.192	119.222	8.364	162.578	252.808	135.778	246.636	90.23	110.858	0.81392412	1.414883158
I+	102.151	3.052	90.357	8.061	152.849	251.948	164.643	246.939	99.099	82.296	1.2041776	2.093279422
IL7												
C-	37.248	3.223	101.707	7.712	217.752	251.777	153.293	247.288	34.025	93.995	0.36198734	0.629259876
C+	120.233	4.634	104.121	9.453	134.767	250.366	150.879	245.547	115.599	94.668	1.221099	2.122694693
P+	137.113	5.469	79.13	7.534	117.887	249.531	175.87	247.466	131.644	71.596	1.83870607	3.196310559
D+	106.625	5.55	104.593	8.94	148.375	249.45	150.407	246.06	101.075	95.653	1.05668406	1.836884347
N+	104.878	7.053	99.255	9.077	150.122	247.947	155.745	245.923	97.825	90.178	1.08479895	1.885757816
I+	118.849	3.018	66.471	8.263	136.151	251.982	188.529	246.737	115.831	58.208	1.98994984	3.459224812

pSTAT-5												
Sample	Mean Gray	Mean Gray Background	Loading Mean Gray	Loading Mean Gray Background	Inverted Mean Gray	Inverted Mean Gray Background	Inverted Loading Mean Gray	Inverted Loading Mean Gray Background	Net Protein	Net Control	Ratio	Normalized Ratio
no IL7												
C-	21.578	6.393	118.092	9.746	233.422	248.607	136.908	245.254	15.185	108.346	0.14015284	1
C+	20.959	5.547	110.358	9.498	234.041	249.453	144.642	245.502	15.412	100.86	0.15280587	1.090280194
P+	20.996	5.779	98.925	8.718	234.004	249.221	156.075	246.282	15.217	90.207	0.16868979	1.203613047
D+	19.067	5.352	118.494	9.451	235.933	249.648	136.506	245.549	13.715	109.043	0.12577607	0.897420749
N+	15.631	5.087	119.222	8.364	239.369	249.913	135.778	246.636	10.544	110.858	0.09511267	0.678635297
I+	17.19	5.012	90.357	8.061	237.81	249.988	164.643	246.939	12.178	82.296	0.14797803	1.055833236
IL7												
C-	151.124	4.098	101.707	7.712	103.876	250.902	153.293	247.288	147.026	93.995	1.56418958	11.16059827
C+	117.153	4.651	104.121	9.453	137.847	250.349	150.879	245.547	112.502	94.668	1.18838467	8.479204842
P+	136.548	4.681	79.13	7.534	118.452	250.319	175.87	247.466	131.867	71.596	1.84182077	13.14151553
D+	136.758	6.012	104.593	8.94	118.242	248.988	150.407	246.06	130.746	95.653	1.3668782	9.752768188
N+	128.689	4.708	99.255	9.077	126.311	250.292	155.745	245.923	123.981	90.178	1.37484752	9.809629886
I+	125.117	6.894	66.471	8.263	129.883	248.106	188.529	246.737	118.223	58.208	2.03104384	14.49163492

A drug treatment killing assay was performed with Jurkat cells, and IL7 was added to the cells for 3 days. The samples were blotted and imaged. Ponatinib and dasatinib treatments show rescue of pLCK and pSTAT5 with the addition of IL7. Refer to Key (above Supplementary Table 6) for sample conditions.

Supplementary Table 11. Analyzed data for pLCK (Top) and pSTAT-5 (Bottom) in IL-rescue western blot with IL15 after four hours of treatment.

pLCK												
Sample	Mean Gray	Mean Gray Background	Loading Mean Gray	Loading Mean Gray Background	Inverted Mean Gray	Inverted Mean Gray Background	Inverted Loading Mean Gray	Inverted Loading Mean Gray Background	Net Protein	Net Control	Ratio	Normalized Ratio
no IL15												
C-	59.578	0.116	62.943	0.062	195.422	254.884	192.057	254.938	59.462	62.881	0.94562746	1
C+	89.703	0.175	38.29	0.282	165.297	254.825	216.71	254.718	89.528	38.008	2.3555041	2.49094302
P+	24.073	0.185	70.757	1.845	230.927	254.815	184.243	253.155	23.888	68.912	0.346645	0.3665767
D+	0	0	71.54	2.719	255	255	183.46	252.281	0	68.821	0	0
N+	92.999	0.373	72.095	0.861	162.001	254.627	182.905	254.139	92.626	71.234	1.30030603	1.37507221
I+	104.452	0.296	46.886	0.303	150.548	254.704	208.114	254.697	104.156	46.583	2.23592298	2.3644861
IL15												
C-	19.276	2.312	44.681	2.459	235.724	252.688	210.319	252.541	16.964	42.222	0.40178106	0.42488303
C+	105.572	5.006	32.422	0.392	149.428	249.994	222.578	254.608	100.566	32.03	3.13974399	3.32027584
P+	33.174	3.779	46.223	2.159	221.826	251.221	208.777	252.841	29.395	44.064	0.66709786	0.70545526
D+	90.011	5.77	41.862	1.177	164.989	249.23	213.138	253.823	84.241	40.685	2.07056655	2.18962186
N+	71.624	3.026	37.011	1.208	183.376	251.974	217.989	253.792	68.598	35.803	1.91598469	2.02615172
I+	106.806	2.309	56.062	3.838	148.194	252.691	198.938	251.162	104.497	52.224	2.00093827	2.11599003

pSTAT-5												
Sample	Mean Gray	Mean Gray Background	Loading Mean Gray	Loading Mean Gray Background	Inverted Mean Gray	Inverted Mean Gray Background	Inverted Loading Mean Gray	Inverted Loading Mean Gray Background	Net Protein	Net Control	Ratio	Normalized Ratio
no IL15												
C-	46.915	3.64	62.943	0.062	208.085	251.36	192.057	254.938	43.275	62.881	0.6882047	1
C+	94.893	5.178	38.29	0.282	160.107	249.822	216.71	254.718	89.715	38.008	2.36042412	3.42982852
P+	86.225	15.042	70.757	1.845	168.775	239.958	184.243	253.155	71.183	68.912	1.03295507	1.5009416
D+	80.175	4.292	71.54	2.719	174.825	250.708	183.46	252.281	75.883	68.821	1.10261403	1.60215997
N+	73.269	3.809	72.095	0.861	181.731	251.191	182.905	254.139	69.46	71.234	0.97509616	1.41686936
I+	69.164	3.439	46.886	0.303	185.836	251.561	208.114	254.697	65.725	46.583	1.41092244	2.05014937
IL15												
C-	105.077	8.859	44.681	2.459	149.923	246.141	210.319	252.541	96.218	42.222	2.27885936	3.31131035
C+	106.606	14.921	32.422	0.392	148.394	240.079	222.578	254.608	91.685	32.03	2.86247268	4.15933321
P+	99.237	31.235	46.223	2.159	155.763	223.765	208.777	252.841	68.002	44.064	1.54325527	2.24243638
D+	79.707	10.852	41.862	1.177	175.293	244.148	213.138	253.823	68.855	40.685	1.69239277	2.45914154
N+	133.636	12.87	37.011	1.208	121.364	242.13	217.989	253.792	120.766	35.803	3.3730693	4.9012587
I+	103.987	5.897	56.062	3.838	151.013	249.103	198.938	251.162	98.09	52.224	1.87825521	2.72921007

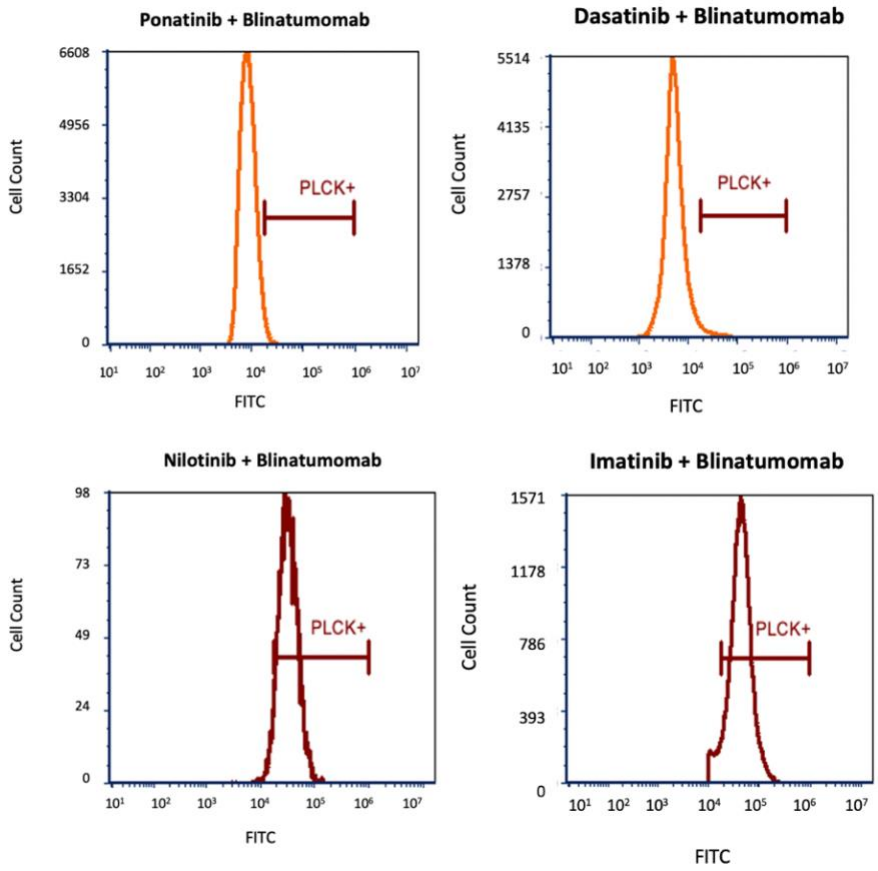
A drug treatment killing assay was performed with Jurkat cells, and IL15 was added to the cells for 3 days. The samples were blotted and imaged. Ponatinib and dasatinib treatments show rescue of pLCK and pSTAT5 with the addition of IL15. Refer to Key (above Supplementary Table 6) for sample conditions.

Supplementary Table 12. Analyzed data for pLCK (Top) and pSTAT-5 (Bottom) in IL-rescue western blot with ILC after four hours of treatment.

pLCK												
Sample	Mean Gray	Mean Gray Background	Loading Mean Gray	Loading Mean Gray Background	Inverted Mean Gray	Inverted Mean Gray Background	Inverted Loading Mean Gray	Inverted Loading Mean Gray Background	Net Protein	Net Control	Ratio	Normalized Ratio
no ILC												
C-	30.34	14.407	87.896	21.109	224.66	240.593	167.104	233.891	15.933	66.787	0.23856439	1
C+	61.469	20.687	76.981	15.818	193.531	234.313	178.019	239.182	40.782	61.163	0.66677567	2.794950501
P+	33.847	12.847	84.795	13.741	221.153	242.153	170.205	241.259	21	71.054	0.29554986	1.238868307
D+	13.382	8.915	73.565	11.677	241.618	246.085	181.435	243.323	4.467	61.888	0.07217877	0.302554686
N+	50.852	16.586	74.372	17.581	204.148	238.414	180.628	237.419	34.266	56.791	0.60337025	2.529171471
I+	50.136	17.759	85.24	13.501	204.864	237.241	169.76	241.499	32.377	71.739	0.45131658	1.89180194
ILC												
C-	108.317	8.65	34.919	7.889	146.683	246.35	220.081	247.111	99.667	27.03	3.6872734	15.45609292
C+	110.33	7.674	43.302	13.058	144.67	247.326	211.698	241.942	102.656	30.244	3.39426002	14.22785689
P+	87.959	13.088	50.082	12.332	167.041	241.912	204.918	242.668	74.871	37.75	1.98333775	8.313636992
D+	71.962	14.107	54.315	13.599	183.038	240.893	200.685	241.401	57.855	40.716	1.42094017	5.956212339
N+	90.828	8.551	76.578	20.04	164.172	246.449	178.422	234.96	82.277	56.538	1.45525134	6.100035834
I+	73.584	14.63	72.786	20.338	181.416	240.37	182.214	234.662	58.954	52.448	1.12404667	4.711711873

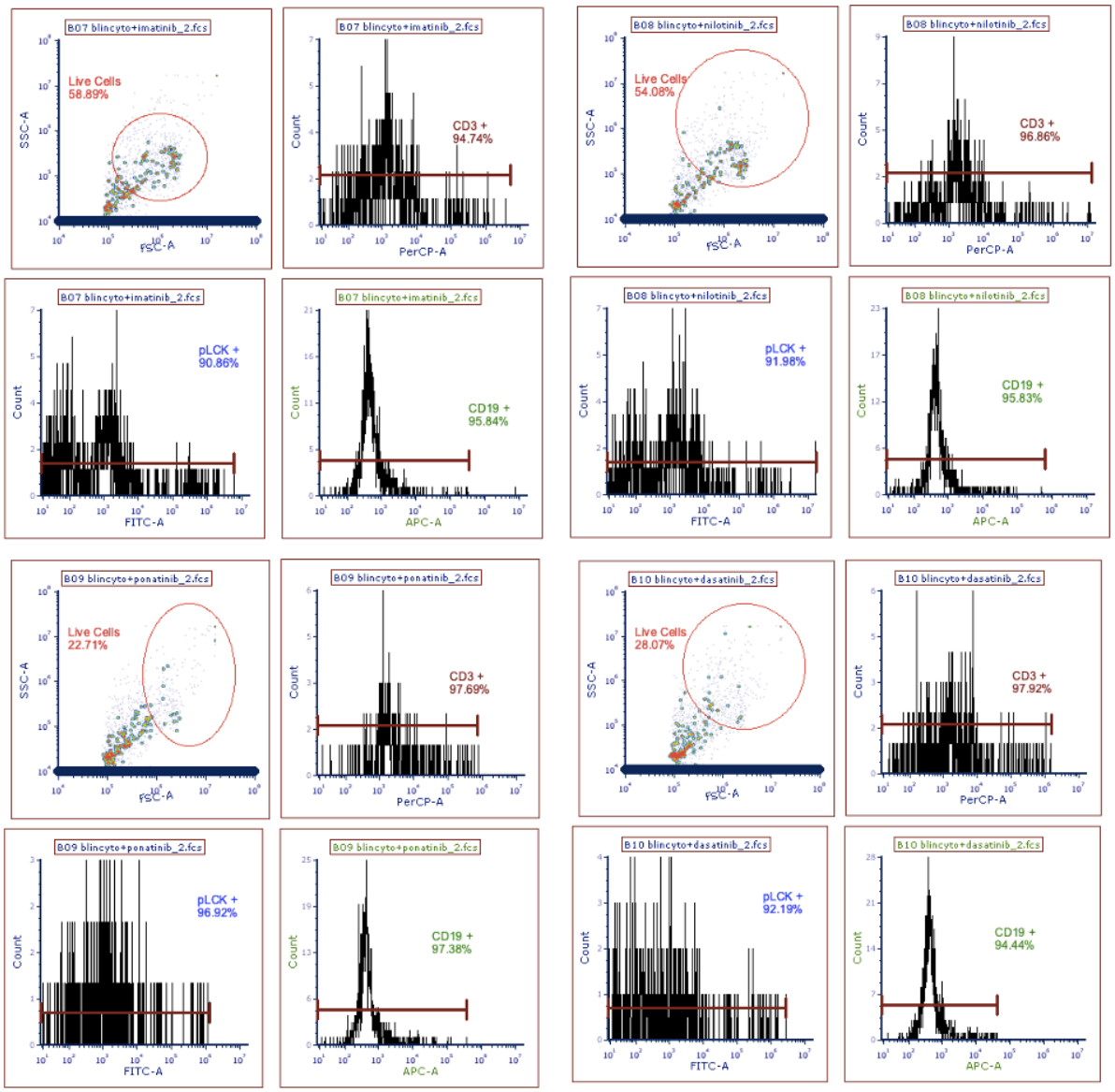
pSTAT-5												
Sample	Mean Gray	Mean Gray Background	Loading Mean Gray	Loading Mean Gray Background	Inverted Mean Gray	Inverted Mean Gray Background	Inverted Loading Mean Gray	Inverted Loading Mean Gray Background	Net Protein	Net Control	Ratio	Normalized Ratio
no ILC												
C-	30.34	0	87.896	21.109	224.66	255	167.104	233.891	30.34	66.787	0.45428002	1
C+	61.469	0	56.981	15.818	193.531	255	198.019	239.182	61.469	41.163	1.4933071	3.287195156
P+	33.847	0.276	84.795	13.741	221.153	254.724	170.205	241.259	33.571	71.054	0.47247164	1.040044941
D+	13.382	0	73.565	11.677	241.618	255	181.435	243.323	13.382	61.888	0.21622932	0.475982446
N+	50.852	0	74.372	17.581	204.148	255	180.628	237.419	50.852	56.791	0.89542357	1.971082861
I+	50.136	0	85.24	13.501	204.864	255	169.76	241.499	50.136	71.739	0.69886673	1.538405141
ILC												
C-	64.628	0.354	34.919	7.889	190.372	254.646	220.081	247.111	64.274	27.03	2.37787643	5.234384752
C+	51.05	0.346	63.302	13.058	203.95	254.654	191.698	241.942	50.704	50.244	1.00915532	2.221438909
P+	54.375	0.368	50.082	12.332	200.625	254.632	204.918	242.668	54.007	37.75	1.43064901	3.149266816
D+	68.192	1.655	74.315	13.599	186.808	253.345	180.685	241.401	66.537	60.716	1.09587259	2.412328361
N+	85.154	1.593	96.578	20.04	169.846	253.407	158.422	234.96	83.561	76.538	1.09175834	2.403271734
I+	79.869	3.17	82.786	20.338	175.131	251.83	172.214	234.662	76.699	62.448	1.22820587	2.703631683

A drug treatment killing assay was performed with Jurkat cells, and ILC was added to the cells for 3 days. The samples were blotted and imaged. Ponatinib and dasatinib treatments show rescue of pLCK and pSTAT5 with the addition of ILC. Refer to Key (above Supplementary Table 6) for sample conditions.



Supplementary Figure 1. FITC (pLCK+) cell counts in BV173 + Jurkat cocultures treated with TKI + blinatumomab combination therapies.

A combination drug treatment kill assay was performed with BV173+Jurkat cocultures. The cells were treated for 2 hours and stained with Per-CP (CD3+) and FITC (pLCK) antibodies. Combinations of SRC TKIs (ponatinib/dasatinib) with blinatumomab showed inhibition of LCK phosphorylation in CD3+ cells. Combinations of non-SRC TKIs (nilotinib/imatinib) with blinatumomab showed phosphorylation of LCK in CD3+ cells.



Supplementary Figure 2. Raw data from pLCK signaling experiment with BV173 + PBMC cells.

A combination drug treatment kill assay was performed with BV173+PBMC cocultures. The cells were treated for 2 hours and stained with APC (CD19+), Per-CP (CD3+), and FITC (pLCK) antibodies. Results were inconclusive due to low PBMC count and fixation error. Results were used for optimization of experiment. Protocol was modified to increase cell counts and add PFA for fixation faster for future replicates.

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ACADEMIC VITA

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Education

Schreyer Honors College, The Penn State University **University Park, PA**
Bachelor of Science in Psychology Neuroscience (pre-medical) May 2022
Minor and Honors in Biology

Research & Select Teaching Experiences at Penn State

Undergraduate Researcher, College of Engineering **Feb. 2020 – Present**

- Studied combinational treatments for Acute Lymphoblastic Leukemia, supervised by Dr. Cheng Dong and Dr. Justin Pritchard
- Developed protocols for laboratory practices: cell culture and counting, western blotting, intracellular staining, and flow cytometry
- Performed two-day, 25-hour bi-weekly experiments to synthesize and collect results using fluorescent-activated cell sorting
- Collaborated with graduate and undergraduate researchers to create computational simulations for cellular growth and decay rates
- Created and presented written data analysis to two biomedical engineering research teams (20 people)

Teaching Assistant, Eberly College of Science

Advanced Human Anatomy: Cadaver-Based **Jan. 2021 – Dec. 2021**

- Promoted student engagement (16 undergraduates) in understanding of clinical applications for anatomical abnormalities

Cadaver Dissection **Jun. 2021 – Aug. 2021**

- Instructed 16 medical, graduate, and undergraduate students in cadaver dissection, under direction of Danielle Waters
- Developed protocols for student dissection and assisted faculty in creating laboratory manual

Introduction to Anatomy and Physiology **Aug. 2020 – May 2021**

- Coordinated learning for 36 undergraduate students to enhance understanding of applied anatomy
- Evaluated and provided feedback on weekly quizzes, exams, and reports

Select Work and Volunteer Experiences

Barber National Institute, Erie, PA
Agency with Choice Caregiver **Aug. 2019 – Present**

- Aided clients with intellectual, physical disabilities with daily tasks and accompanied them on various outings

Recreation Assistant at Camp Shamrock **Jun. 2019 – Aug. 2019**

- Constructed programming and maintained a positive environment at a camp for children with intellectual, physical disabilities

Camp Kesem Central PA (CKCPA), State College, PA
Outreach Coordinator **Aug. 2021 – Present**

- Maintained year-long communication between CKCPA and camper families as primary point-of-contact
- Initiated, developed, and retained partnerships with community organizations and key stakeholders
- Recruited new campers by effectively communicating organization's mission and leveraging connections with community partners
- Created and managed year-round fundraising initiatives; solicited donations from community supporters

Camp Counselor **Oct. 2019 – Present**

- Collaborated with 150 volunteers to coordinate a free, week-long summer camp for children with parents affected by cancer

Skills

- American Sign Language, conversational
- *MS Office* (Word, PowerPoint, Excel)
- Mac proficiency
- MiniTab
- SPSS Software
- FCS Express