THE EFFECTS OF COMMONLY USED ANTIBIOTIC MEDICATIONS ON THE EFFICACY OF TYROSINE KINASE INHIBITORS IN PHILADELPHIA CHROMOSOME POSITIVE LEUKEMIA

by

Brooke Marie Zhao

A thesis submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

in

Laboratory Medicine and Biomedical Science

Department of Pathology

The University of Utah

December 2017

The University of Utah Graduate School

STATEMENT OF THESIS APPROVAL

The thesis of **Brooke Marie Zhao**

has been approved by the following supervisory committee members:

Elena Y. Enioutina	, Chair	**7/27/17** Date Approved
Diana G. Wilkins	, Member	**7/27/17** Date Approved
Jonathan Constance	, Member	**7/27/17** Date Approved
Jessica C. Brown	, Member	**7/27/17**
Janis Weis	, Member	**7/27/17**

and by **Peter E. Jensen**, Chair/Dean of

the Department/College/School of **Pathology**

and by David B. Kieda, Dean of The Graduate School.

ABSTRACT

The main purpose of this thesis project is to develop and validate a high-throughput protocol to test drug-drug interactions. As a model, tyrosine kinase inhibitors were tested with commonly used antibiotics in Philadelphia chromosome positive leukemia patients. Tyrosine kinase inhibitors (TKI's), such as imatinib mesylate and dasatinib, are currently the frontline therapeutic agents for patients with leukemia characterized with the presence of the Philadelphia chromosome (Ph^+), generated through a translocation event between chromosomes 9 and 22 (t[9,22][q34;q11]). However, interactions between TKI's and medications commonly taken by patients with leukemia, including antibiotics, have not been significantly studied. In this thesis, a protocol was established using innovative, high-throughput technology to determine imatinib and dasatinib maximal inhibitory concentrations and possible interactions with drugs that are often administered concomitantly with TKI therapy, antibiotics in particular. K562 cells were cultured in the presence of TKI's and cell proliferation was measured. Inhibitory concentrations of the TKI's were determined. Imatinib at concentrations IC_{10}, IC_{50}, and IC_{90} were then co-incubated with co-medications at concentrations reflecting therapeutic exposure. Co-medications were gentamicin, ceftazidime, sulfamethoxazole, trimethoprim, and a combination of sulfamethoxazole and trimethoprim at a 1:23 ratio, respectively, to emulate the constituents of the antibiotic commonly known as Bactrim. The results determined that sulfamethoxazole and trimethoprim have no significant effect

on K562 cell proliferation or the ability of imatinib to reduce K562 cell proliferation. Gentamicin and ceftazidime displayed no difference from control perturbation on imatinib efficacy. However, gentamicin and ceftazidime were found to have an effect on K562 proliferation without the addition of a TKI. Therefore, further investigation is necessary to understand the mechanism behind gentamicin and ceftazidime reducing K562 cell proliferation. This assay can also be used to assess for potential *in vitro* TKI-drug interactions using robust, high-throughput screening techniques.

TABLE OF CONTENTS

LIST OF FIGURES

Figures

INTRODUCTION

The Philadelphia Chromosome and *BCR-ABL*

In 1960, a junior faculty member at the University of Pennsylvania School of Medicine, Peter C. Nowell, and a graduate student, David Hungerford, reported a minute chromosome in seven patients with chronic myelogenous leukemia (CML).[1] This unusually small chromosome was designated the "Philadelphia chromosome" after the city in which it was discovered.[1] The Philadelphia (Ph) chromosome was the first evidence that provided support to the hypothesis that a genetic alteration could give rise to a tumor from abnormal cell growth.[1,2] Later, as cytogenetic technology improved, the Philadelphia chromosome was determined to be a translocation between chromosomes 9 and 22 that resulted in a gene fusion of the Abelson murine leukemia or c-*abl* oncogene from chromosome 9 to the breakpoint cluster region or *bcr* on chromosome 22 [t(9;22)(q34;q11)].[2–4] This fusion gene encodes the product BCR-ABL1, which is a constitutively active tyrosine kinase.[4]

The breakpoints in the *bcr* and *abl1* regions are highly variable, but the recombination generally involves *bcr* intron 1, intron 13/14, or exon 19 fusing with *abl1* between exons 1b and 2.[4,5] These fusions create three different sized proteins coded as p210, p190, and p230.[4] p210 is most commonly detected in chronic myelogenous leukemia (CML) and occasionally acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML).[4] *Bcr-abl* p190 is commonly detected in B-cell ALL but

rarely in CML.[4] Researchers have discovered that p190 and p210 are formed by different mechanisms. Specifically, breakpoints in ALL with p190 *bcr-abl* suggest that activity of the recombinase-activating gene (RAG) is involved whereas there is no indication of RAG activity in CML p210 cases.[5] p230 is also known as the μ *bcr-abl1* transcript, generated by a fusion of almost the entire *bcr* and *abl1* genes, and is considered a molecular diagnostic marker for neutrophilic-chronic myeloid leukemia (CML-N).[4]

Bcr-abl1 is a complex fusion oncogene with multiple domains arising from both the BCR and ABL1 regions.[4,6] The domains from ABL1 include src homology domains (SH1/SH2), which contain an autophosphorylation site that dictates the switch between the active and inactive kinase conformation, a proline-rich domain, and DNA- and actin-binding site domains.[4,7] The BCR domains include an N-terminal coiled-coil (CC) domain and a Ser/Thr kinase domain (Y177). The N-terminal CC is essential and sufficient to activate the ABL1 kinase to induce a myeloproliferative disorder (MPD) in mice and the Y177 domain made the induction of MPD more efficient by assisting in leukemic cell progenitor expansion, proliferation, and survival.[4,8] Another domain within BCR is a RAS homolog gene family/Guanine nucleotide exchange factors (Rho/GEF) kinase domain.[9] Differential activation of the Rho proteins plays a major role in BCR-ABL1-induced leukemogenesis and the phenotype expressed.[9] BCR-ABL1 is located in the cytoplasm, which is a prime position to disrupt multiple cystolic and membrane signaling pathways.[6]

The BCR-ABL1 kinase activity inhibits differentiation, stimulates proliferation, and blocks cell death.[4] Hyperactivity of this kinase results in activation of signaling pathways and interference of normal cellular processes.[4] For instance, the JAK/STAT

pathway, which is required for disease initiation, is activated to promote cell growth/survival and control BCR-ABL1 stability.[10–12] The PI3K-AKT-mammalian target of rapamycin (mTOR) pathway is another important downstream cascade in Ph^+ leukemia because it allows BCR-ABL1 to evade cell cycle arrest.[12,13] Activation of the RAS/RAF/MEK/ERK pathway and the downregulation of the death receptor ligand, TRAIL, prevents apoptosis and results in uncontrolled cell proliferation.[4,12] BCR-ABL1 activity also enhances the expression of the *WRN* gene to promote genomic instability.[4] The BCR-ABL1 kinase uses these cell pathways to deregulate normal cellular activity; however, variation in the expression patterns of BCR-ABL leads to differences in leukemia phenotype and phase or progression of the leukemia.

Chronic Myelogenous Leukemia

Chronic myelogenous leukemia (CML) is often the myeloproliferative disorder characterized by the presence of Philadelphia chromosome. The gene translocation t(9;22)(q34;q11) is seen in up to 90% of adult CML cases and is the primary cause for the initial phase of CML.[6,14] This form of leukemia is distinguished by the increased and unregulated growth of mainly myeloid cells or, less commonly, lymphoid cells in the bone marrow and blood.[4,6] Major symptoms that are associated with CML include fatigue, anemia, splenomegaly, episodes of infections, and abdominal discomfort.[15] On a complete blood count (CBC), CML is suspected when there is an increase of granulocytes of all types including basophils and eosinophils.[6]

Worldwide, CML makes up about 15-20% of all leukemias with incidence of 0.7-1.8/100,000 population, depending on the study considered.[6,16] CML is uncommon

among children and adolescents: CML constitutes 2% of all leukemias in children younger than 15 and 9% of all leukemias in adolescents between 15 and 19 years.[17] Familial occurrence of CML is rare and most patients have no history of increased exposure to radiation, but the incidence does rise with exposure to very high levels of radiation.[6] Survivors of the nuclear bomb explosions in Japan were shown to have an incidence of CML that was approximately 50 times higher than subjects that weren't exposed to radiation about 10 years after the explosion.[6] Other risk factors include gender (CML is slightly more common in males than females), increasing age, and obesity.[18,19]

Untreated CML often progresses through three stages: the chronic phase (CML-CP), the accelerated phase (CML-AP), and the fatal blast crisis phase (CML-BC).[4] Diagnosis is generally made during the CML-CP and the duration of this phase is variable depending on when the diagnosis was made.[20] The World Health Organization (WHO) defined the criteria for the phases to be the blasts percent of peripheral blood white cells or bone marrow cells: CML-CP is less than 10%, CML-AP is 10%-19%, and CML-BC is 20% or more.[21]

Most CML patients have the p210 *bcr-abl* and occasionally a low level of p190 *bcr-abl*; patients with the presence of two Ph^+ breakpoints generally have a worse prognosis than those with a single breakpoint.[4,22]

Without therapeutic intervention, CML would progress from CML-CP to CML-BC through the accelerated phase and the median survival was three years.[15] The first treatments that were used for patients with CML was interferon-α (IFN-α) therapy and stem cell transplantation.[15,23] Interferon-α therapy doubled the median survival to six years, and patients that underwent stem cell therapy under optimal conditions have a

31.5% chance of living up to ten years.[24,25] However, the greatest improvement in therapy came when tyrosine kinase inhibitors (TKIs) were discovered, which has radically improved patient outcome significantly. Since the development of imatinib treatment, the mortality rate is not statistically significantly different than the general population.[26] A 2011 prospective study found that almost 95% of patients that were treated with imatinib had a complete cytogenetic response (no Ph^+ cells can be measured) six years after starting therapy.[26] Despite these exceptional advances in CML therapy, 20-25% of patients experience imatinib resistance, mostly due to mutations in the BCR-ABL1 protein.[27] Second-generation TKIs and targeting different cellular pathways have helped circumvent resistance and research of future treatment options is continuous in order to improve the survival of patients with CML.

Acute Lymphoblastic Leukemia

There are an estimated 6,000 new cases of Acute Lymphoblastic Leukemia (ALL) in the United States annually.[28] While ALL occurs in both children and adults, approximately 60% of patients are <20 years of age and ALL is known as the most common childhood cancer.[28,29] The Philadelphia chromosome can also be found in some patients with acute lymphoblastic leukemia (ALL). Approximately 20-30% of adult ALL patients and 3-4% of pediatric ALL patients have this translocation that leads to the creation of the BCR-ABL1 kinase.[30,31] ALL is characterized by the accumulation of lymphoblasts (immature white blood cells) in the bone marrow.[32] Major symptoms that are associated with ALL are similar to patients with CML and they include enlarged lymph nodes, splenomegaly, fatigue, bone or joint pain, and enlarged thymus.[33] The

clinical presentation of Ph^+ ALL is indistinguishable from ALL with other abnormalities and diagnosis requires cytogenetic or fluorescence *in situ* hybridization (FISH) detection of the *bcr-abl1* translocation.[30]

Similar to CML, ionizing radiation is an established causal exposure for childhood ALL, but etiology is often not established.[28] An abnormal response to common infections and Down's Syndrome are other factors that have shown to correlate with ALL; however, it is difficult to determine relevant exposures or genetic variants because of the many biologically distinct subtypes of ALL.[28]

ALL is classified into different subtypes based on hematopoietic origin of leukemic cells and degree of cell maturation.[34] B-cell ALL is a neoplasm of B-cell precursors (B-lymphocytes) and T-cell ALL is characterized by expansion of immature T-lymphocytes. Twenty to 30% of adult B-cell ALL cases involve the Philadelphia chromosome, but Ph^+ T-cell ALL is rare and only a few cases have been reported in the literature.[35]

Ph^+ ALL patients mostly express the p190 *bcr-abl1* transcripts, which only retains the first exon of *BCR*.[4,30] A larger protein, with the p210 breakpoint, is seen in 30% of adult and 20% of childhood patients with Ph^+ ALL.[4] The p190 breakpoint has shown to have stronger transforming activity compared to p210, which could be due to a broader substrate range in the p190 protein.[30]

Prior to the use of tyrosine kinase inhibitors, chemotherapy without hematopoietic stem cell transplantation (HSCT) showed prognosis for both pediatric and adult patients with Ph^+ ALL as very poor, with less than 5% of adults being cured.[4,30] The introduction of imatinib showed improvement in response rates and survival; 73% of adult patients

had a significant reduction in marrow or peripheral blasts when treated with imatinib and the 3-year event free survival (EFS) rate for pediatric patients went from 35% to 80% when treated with a combination therapy of imatinib and chemotherapy.[30,36] However, survival rates for children and adults with Ph^+ ALL are still much lower compared to other types of ALL.[30,37] More research needs to be done to determine the effectiveness of combined HSCT, chemotherapy, and/or TKI therapies for both children and adults.[36]

Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is also seen with the Philadelphia chromosome translocation. AML is the most common acute leukemia in adults, accounting for approximately 80% of leukemia cases, and constitutes 20% of pediatric leukemia cases.[38,39] However, the *bcr-abl1* transcripts are seen in less than 1.5% of patients with AML.[4]

AML is a type of cancer that is characterized with abnormal myeloblasts, red blood cells, or platelets development.[40] When the Philadelphia chromosome is detected in patients with AML, it is often cytogenetically indistinguishable between CML-BC.[4,41] Despite the similarities, Ph^+ AML appears with distinct features from CML-BC including fewer cases with splenomegaly, less peripheral basophils, lower bone marrow cellularity, and a lower myeloid/erythroid ratio.[4,41,42] Additionally, the breakpoint on chromosome 22 differs between CML in approximately 50% of the cases.[4] The p210 and p190 breakpoints can both be observed in AML patients, whereas the p190 breakpoint is rarely observed in CML.[4] Research indicates that there is a higher rate of relapse with AML patients that have the p190 breakpoint.[43]

The median survival time of Ph^+ AML is 9 months, which is very similar to the 7-month median survival of myeloid CML-BC patients.[4] Effective therapeutic strategies are lacking. However, survival improved with the implementation of tyrosine kinase inhibitor therapy, especially when combined with chemotherapy and HSCT, by increasing the median survival rate from 9 months to 18 months.[43]

BCR-ABL1 Tyrosine Kinase Inhibitors

Chronic myeloid leukemia is a unique disease because it appears to be the result of a single biochemical defect and, therefore, the BCR-ABL protein is a logical target for therapeutic intervention. Signal transduction inhibitor-571 (STI-571), also known as imatinib mesylate, Gleevec (United States), or Glivec (Europe), was discovered after researchers searched for a drug that inhibited the BCR-ABL kinase.[44] However, 500 protein kinases were found during studies of the human genome and many researchers and clinicians feared the toxic effects that may occur with the use of a kinase inhibitor.[45] Nevertheless, imatinib received Food & Drug Administration (FDA) approval in May 2001 for the treatment of multiple cancers, including Ph^+ CML.[46] With the success of imatinib, other tyrosine kinase inhibitors have been developed to inhibit different types of mutations and forms of resistance found in patients with Ph^+ leukemia.

First-Generation Tyrosine Kinase Inhibitor

Imatinib is considered a first-generation tyrosine kinase inhibitor because it was the first drug approved to specifically target the BCR-ABL1 tyrosine kinase protein. The BCR-ABL1 tyrosine kinase catalytic domain has two lobes, the N-terminal lobe (N-lobe)

and the C-terminal lobe (C-lobe).[47] In the inactive state, the activation loop, which has a key role in the activation of the kinase, is folded in toward the ATP binding site.[47] Imatinib binds the kinase in the inactive state between the N- and C-lobe and spans most of the kinase's width.[47] Imatinib binds close to the adenosine triphosphate (ATP) binding site, which locks it in a closed conformation.[4] Inhibiting the phosphorylation of ATP prevents the switch to the active or "open" conformation, which blocks downstream signaling pathways.[4,48] Imatinib has a high affinity for the Abl kinase and is inactive against Ser/Thr-kinases and most normal functioning tyrosine kinases.[48] Imatinib also inhibits the c-KIT and PDGFR tyrosine kinases that play a role in gastrointestinal stromal tumor (GIST) formation.[49]

Initial studies with imatinib in rats and dogs demonstrated that bioactive concentrations of imatinib are readily achieved in the circulation.[50] Administration of imatinib 3 times per day, which assured a continuous block of BCR-ABL1 activity, over 11 days cured 87-100% of the treated mice, whereas once or twice per day did not.[51] This study demonstrated that continuous exposure to imatinib is necessary for success. Pharmacokinetic studies demonstrated that the half-life for imatinib is 12-14 hours and that predicted effective concentrations correlated well with preclinical trials.[50] Currently, clinical guidelines recommend frontline treatment of Ph^+ CML-CP to be oral administration of 400 mg/day and 600 mg/day if the disease has progressed to CML-AP or CML-BC.[52] These dosages are similar for other Ph^+ leukemias including Ph^+ ALL.[52] Some side effects of imatinib treatment include rash, weight gain, edema, pleural effusion, cardiac toxicity, nausea and vomiting, arthralgias and myalgias, and myelosuppression.[53]

The development of imatinib has increased the survival of CML immensely compared to the previous standard of care, IFN-α. In a randomized study comparing ST1571 (imatinib) and Interferon, they showed that imatinib had higher rates of major molecular response, complete cytogenetic response, and long-term and transformation-free survival.[54] In CML-CP, the median 8-year survival rate was ≤15% before 1983, 42%-65% from 1983-2000, and 87% since 2001 – the year that imatinib mesylate was FDA approved.[25] In CML-AP, the 8-year survival is 75% since 2001.[25] The survival of patients in CML-BC has only modestly improved since the introduction of imatinib: the median survival went from 6 months to 7 months since 2001.[25]

Despite the success of imatinib treatment, 33% of patients have shown to have an unfavorable outcome primarily because they become resistant to this therapy.[55] Patients required monitoring while being treated with imatinib to identify failure and suboptimal responses. Patients classified with failure and suboptimal response undergo dose escalation.[55,56] Patients who had a failed response and then increased their daily dose of imatinib from 400 mg to 600-800 mg showed to have a significant improvement.[55] In a recent study investigating imatinib dose escalation, 42% of patients who had their dose escalated obtained a cytogenetic response within 12 months.[57] While dose escalation is a viable treatment option for patients after failure of the standard dose of imatinib, it is not effective for all patients and the use of second-generation TKIs is also recommended.

Second-Generation Tyrosine Kinase Inhibitors

Mechanisms of resistance to imatinib have been extensively investigated, which lead to the development of second-generation TKIs. The first drug approved by the FDA

as a second-line treatment for Ph^+ CML-CP, -AP, and –BC was dasatinib in 2006.[55] Dasatinib (Sprycel) has a different chemical structure compared to imatinib, which binds the inactive and active forms of BCR-ABL1 and has greater activity against BCR-ABL1 compared to imatinib.[58,59] Dasatinib also has inhibitory activity against many of the imatinib-resistant BCR-ABL1 mutations.[60]

The START-C trial, a multicenter, phase 2 trial, assessed patients with CML-CP who demonstrated imatinib resistance or intolerance and initiated a twice-daily dasatinib regimen.[60] This trial found that 62% of patients obtained major cytogenetic response and 53% had a complete cytogenetic response, which confirmed the use of dasatinib treatment to treat relapse or resistant CML.[60] The START-R trial compared dasatinib treatment to high-dose imatinib treatment and observed a higher percentage of patients treated with dasatinib achieving a complete cytogenetic response compared to imatinib, 44% and 18%, respectively.[55,61] Additionally, dasatinib has been reported to be effective against CML-AP and –BC. The START-A trial demonstrated complete cytogenetic responses were achieved in 32% of patients with CML-AP.[62] Complete cytogenetic responses in CML-BC patients differed depending on the whether patients had myeloid blast phase (26%) or lymphoid blast phase (46%) CML.[63]

The recommended starting dose for patients with Ph^+ CML-CP is 100 mg once daily and 140 mg once daily for patients with CML-AP or –BC.[58] Some common side effects that impact approximately 30% of patients include low blood counts, fluid retention (pleural effusion or pericardial effusion occur in about 9% of cases), diarrhea, headache, bleeding, muscle and bone pain, fatigue, fever, rash, nausea, and infection.[64]

Another second-generation TKI, nilotinib (Tasigna), was approved to use as

second-line treatment for CML in 2007.[58] Nilotinib results from modifications to the imatinib molecule to create more favorable interactions and a better fit into the hydrophobic ATP binding space of the inactive conformation of BCR-ABL1.[65] These changes resulted in a drug 30 times more potent than imatinib with increased selectivity for the ABL protein and less cross reactivity with other molecules including PDGFR and KIT.[55,65]

The recommended dosing schedule for nilotinib is 400 mg twice daily, which was approved based on a Phase 2 study with CML-CP and –AP patients who failed initial imatinib therapy.[58] This study showed that 46% of these patients had a complete cytogenetic response when given nilotinib.[58] However, the efficacy of nilotinib appears to be lower in advanced disease. Only 19% of patients with CML-AP showed a complete cytogenetic response and only 25% of CML-BC patients achieved a complete hematologic response.[58]

The most common adverse events seen with nilotinib include bone marrow suppression (neutropenia, thrombocytopenia, and anemia) and asymptomatic serum lipase elevations.[58] Cardiac adverse events are also more common with nilotinib than imatinib or dasatinib: QTc-interval prolongation and palpitations have been reported in 1-10% of patients.[58] Other side effects include rash, pruritus, headache, nausea, fatigue, and liver toxicity.[65]

After the failure of imatinib, it is necessary to decide which second-generation TKI would be most effective for each patient. Analysis of imatinib resistant CML-CP patients was performed to understand the impact of various BCR-ABL mutations and the impact of those mutations on dasatinib or nilotinib therapy.[66] Dasatinib was effective

regardless of most mutations; only a subgroup of patients had mutations that caused a less favorable outcome (Q252H, E255K/V, V299L, and F317L).[66] Treatment with nilotinib showed similar results, but the mutations that were less sensitive to nilotinib therapy included E255K/V, Y253F/H, and F359C/V.[66] These studies indicated that most patients have similar responses with dasatinib and nilotinib except for a few particular mutations where treatment should be chosen carefully. Another consideration to take when choosing second-generation TKI treatment is the potential adverse events that may be more pronounced based on the patient's comorbidities. For example, pleural effusion is more common in patients receiving dasatinib treatment, so patients with risk factors for pleural effusion such as hypertension and chronic obstructive pulmonary disease are at greater risk for developing complications.[66]

Subsequent clinical trials of nilotinib and dasatinib have been performed to determine whether second-generation TKIs should be expanded to be included in first-line therapy.[67,68] Although there is limited information on the long-term response rate of these TKIs as first-line therapy, one study evaluated the efficacy and safety of using these drugs for first-line treatment.[68] It was determined that second-generation TKIs can be safely used as therapy for patients with CML-CP.[68]

Third-Generation Tyrosine Kinase Inhibitor

After the failure of imatinib and second-generation TKIs, or in the presence of the T315I mutation that all first- and second-generation TKIs are ineffective against, a third-generation tyrosine kinase inhibitor may be considered. Ponatinib (Iclusig) is an oral third-generation TKI able to inhibit native BCR-ABL1 proteins and most of the clinically

relevant BCR-ABL1 mutations, including the T315I mutation.[69] This drug was developed using a structure-based approach to design a small TKI that can access the ATP binding site of mutated BCR-ABL that block the access of imatinib, dasatinib, and nilotinib.[70]

Ponatinib is currently approved for patients with CML who are resistant or intolerant to previous TKI therapy. A phase 2 trial was conducted and they found that 46% of 267 patients with CML-CP had a complete cytogenetic response (40% of patients with resistance to or unacceptable side effects from dasatinib or nilotinib and 66% of patients with the T315I mutation).[71] There were no BCR-ABL mutations that conferred resistance to ponatinib detected.[71] However, 12% of the patients in this study discontinued use due to adverse events caused by ponatinib including serious arterial thrombotic events.[71] Congestive heart failure, fluid retention, pancreatitis, and hemorrhagic events were also recorded in the patients in the trial.[60] Due to these severe risks associated with ponatinib, this drug is only indicated for patients who are T315I-positive or patients for whom no other TKI is indicated.[67]

Tyrosine Kinase Inhibitor Resistance

Imatinib is considered the standard of care in CML management and has shown to have superior results compared to past therapies. However, approximately one-third of CML patients treated with imatinib do not achieve complete cytogenetic response due to factors including drug resistance.[72] Also, more than 80% of Ph^+ ALL patients have reported resistant mutations at time of relapse.[30] Patients that fail to achieve hematologic, cytogenetic, or molecular responses are considered primarily resistant, and those losing previously obtained milestones are deemed secondarily resistant.[27] The mechanisms of

resistance to imatinib can be either *BCR-ABL* dependent (point mutations or gene amplification) or *BCR-ABL* independent.[72]

Treatment failure is often due to point mutations in the *BCR-ABL* kinase domain. Over 100 different point mutations have been characterized; the most frequent mutations occur in the ATP phosphate-binding activation loop.[72] F317 and T315I were the most common mutations with dasatinib, and Y253H and T315I were predominant with nilotinib.[73] Thus, cross-resistance is limited to T315I, which is known as the gatekeeper mutation, and results when a single threonine to isoleucine nucleotide substitution occurs in the activation loop.[72] The other BCR-ABL-dependent mechanism of resistance is amplification; however, this is a less common mode of treatment failure.[27,72]

BCR-ABL-independent mechanisms of resistance include the increased or decreased expression of drug influx and efflux transporters and other molecules that inhibit the activity of imatinib or other tyrosine kinase inhibitors. Increased expression of the P-glycoprotein (P-gp) drug efflux pump *MDR1* was found in patients with CML, and silencing of *MDR1* by RNAi restored imatinib susceptibility to previously resistant lines.[74] Another study demonstrated that patients with lower levels of the drug uptake human organic cation transporter 1 (hOCT1) led to decreased drug uptake, and when hOCT1 levels were increased, patients had a much higher chance of achieving a major molecular response (MMR).[75] Other factors that cause imatinib resistance include sequestration of imatinib in the plasma by serum protein α1-acid glycoprotein, lower serum imatinib concentration, and alternative signal pathway activation through Ras/Raf/Mek kinase, STAT/JAK, or SFK phosphorylation of BCR-ABL1.[76,77]

Antibiotics

Infection is one of the most common, and serious, complications that can occur in cancer patients and various antibiotics are used to treat this depending on the type of infection. The most common antibiotics used include sulfamethoxazole/trimethoprim, gentamicin, and ceftazidime.

Bactrim (Sulfamethoxazole/Trimethoprim)

Bactrim is a combination of two drugs sulfamethoxazole/trimethoprim known by a variety of names including SMX/TMP, co-trimoxazole, Bactrim, Septra, and Sulfatrim.[78] This medication is active against gram-positive and gram-negative microorganisms.[78] It is administered orally or by an intravenous (IV) fusion.[78] SMX/TMP or Bactrim is approved for use in patients from 2 months of age to adulthood.[78] In both the pediatric and adult cancer population, SMX/TMP is commonly used prophylactically and empirically.[79]

Contraindications of SMX/TMP use include known hypersensitivity to sulfonamides or trimethoprim, documented megaloblastic anemia due to folate deficiency, pregnant/nursing women, and children <2 months of age.[78,80] Some adverse effects have occurred in patients using this medication including body aches, blistering skin, rash, fever, sun-sensitivity, and diarrhea.[81] Other severe warning that are listed on the FDA drug safety label requirements include thrombocytopenia, Stevens-Johnson Syndrome, toxic necrolysis, MRSA development, folate deficiency, hemolysis, and hypoglycemia.[80] Interactions with SMX/TMP have been found with other drugs including antidepressents, antibacterials, antivirals, anticoagulants, diuretics,

cyclosporine, and methotrexate.[78]

The Bactrim tablet is composed of trimethoprim and sulfamethoxazole at a ratio of 1:5 and administered orally or through IV fusion. [78] These medications are then widely distributed into body tissues and fluids.[78] These two drugs are more effective when given together than when given separately because they inhibit successive steps in the folate synthesis pathway.[82] Sulfamethoxazole blocks the synthesis of dihydropteroic acid from para-aminobenzoic acid and pteridine; trimethoprim then acts to inhibit the reduction of dihydrofolic acid to tetrahydrofolic acid by the inhibition of the enzyme dihydrofolate reductase.[82] The inhibition of the biosynthesis of tetrahydrofolic acid interrupts the bacterial synthesis of purines, thymidine, serine, and methionine, which are all essential to the function of microbes.[82] Both sulfamethoxazole and trimethoprim are excreted in urine by glomerular filtration and active tubular secretion approximately 8-13 hours after administration.[78]

Gentamicin

Gentamicin sulfate is an antibacterial medication sold under the brand name Garamycin and is used to treat serious gram-negative bacterial infections.[83] It is administered by IV infusion or intramuscular (IM) injection.[83]

Gentamicin is a commonly used antibiotic routinely given to hospitalized newborns, children, adults, and the elderly.[84,85] Pediatric oncology patients are often given gentamicin as treatment for fever and neutropenia, but the pharmacokinetic profile changes depending on age and chemotherapy treatment, which requires varied dosing parameters.[86] Gentamicin use in adult patients with AML also requires dosage

variation.[87]

Gentamicin has been known to cause ototoxicity, which can lead to temporary or permanent hearing loss and/or loss of balance, and nephrotoxicity, which leads to a buildup of urine and wastes.[83] Gentamicin has also been reported to cause neuromuscular blockade, respiratory paralysis, allergic responses, low blood counts, and fetal nephrotoxicity.[83] Interactions with gentamicin have been found with other drugs including β-lactam antibiotics, carbapenems, chloramphenicol, clindamycin, diuretics, neuromuscular blocking agents, NSAIDs, and tetracyclines.[83]

Gentamicin cannot be absorbed by the gastrointestinal tract and must be given parenterally.[83] The mechanism of action of gentamicin is similar to other aminoglycosides, which work by binding to the bacterial 30S ribosomal subunit, leading to a misreading of the t-RNA, and this leaves bacteria unable to synthesize proteins essential to growth.[88] The gentamicin complex is composed of three main parts, which make up approximately 80% of gentamicin: gentamicin C_1, gentamicin C_{1a}, and gentamicin C_2.[89] These major components mainly differ in the degree of methylation in the 2-amino-hexose ring and the ratio between these components is highly variable depending on the manufacturing process.[90] The remaining 20% is composed of gentamicin groups A and B, gentamicin X, and some others.[89] The half-life of gentamicin is 2-3 hours and is eliminated by glomerular filtration within 24 hours of administration.[83]

Ceftazidime

Ceftazidime is a β-lactam antibiotic and third-generation cephalosporin.[91] It is also known as Fortaz or Tazicef.[91] Ceftazidime is used for the treatment gram-negative

and gram-positive aerobes and anaerobes.[91] It is administered by injection into a vein or muscle.[91] Ceftazidime is commonly used in patients with leukemia to treat bacterial infections, but a higher dose may be necessary to maximize drug exposure.[92]

Some common side effects that may occur while taking ceftazidime include allergic reactions, nausea, and pain at the site of injection. More severe side effects include emergence of *Clostridium difficile*, C. *difficile*-associated diarrhea and colitis, and neurotoxicity.[91] Drug interactions can occur with aminoglycosides, chloramphenicol, probenecid, and glucose tests.[91]

Peak serum concentrations of ceftazidime are attained approximately 1 hour after administration of the dose and becomes widely distributed into body tissues and fluids.[91] Ceftazidime has a high affinity for the enzymes responsible for cell-wall synthesis, especially penicillin-binding proteins (PBP's).[93,94] Ceftazidime causes filamentation and eventual cell lysis of bacteria due to its activity against PBP-3; this suppression also inhibits the bacteria's cell division capabilities.[94] Ceftazidime's half life is 1.4-2 hours and is completely eliminated in the urine by glomerular filtration approximately 24 hours after administration.[91]

Drug Interactions in Cancer Therapy

Polypharmacy is commonly defined as the concomitant use of five or more drugs.[95] Polypharmacy is associated with an increased risk of drug-drug interactions leading to a decrease or potentiation of a drug's efficacy and/or adverse health outcomes.[95]

Drug-drug interactions can be classified as pharmacokinetic or pharmacodynamic.

Pharmacokinetics investigates the movement of the drug in the body including the processes of absorption, distribution, metabolism, and excretion.[96] Pharmacodynamics refers to an observed effect at the site of action resulting from a certain drug concentration and can occur when two drugs with similar mechanisms of action are combined.[96] Pharmacokinetic interactions can occur at any site in the body that involves absorption, distribution, metabolism, or excretion, whereas pharmacodynamic interactions happen on pharmacological receptors, signal transduction mechanisms, or physiological systems.[97] Consequences of pharmacokinetic or pharmacodynamic interactions are described as synergy, potentiation, or antagonism. Polypharmacy is common among the elderly and patient populations who are likely to be prescribed many medications, including cancer patients.

Cancer patients are particularly susceptible to drug-drug interactions because they often receive multiple medications – along with drugs to treat comorbid conditions, patients receive medications to treat cancer-related symptoms or therapy-induced toxicity including pain and infection.[98] In a study examining 405 cancer patients, one-third of the patients were exposed to at least one drug-drug interaction and 86% of these interactions were classified as major or moderate.[98] Additionally, the risk factor for potential drug interactions increased with an increased number of medications.[98]

Imatinib has been shown to have interactions with specific drugs, even though the interactions with many drugs that CML patients frequently take are still unknown. Imatinib is mainly metabolized by the cytochrome p450 isoenzyme 3A4 and is a substrate of the human organic cation transporter type 1 (hOCT1), P-glycoprotein (Pgp), and the breast cancer resistance protein (BCRP).[99] Therefore, when inhibitors of the

CYP3A family are given, such as levothyroxine, voriconazole, or amiadorone, there are pharmacokinetic interactions that can alter the plasma levels of imatinib.[99–102] The pharmacokinetic profile of imatinib was also significantly altered by CYP3A4 inducers like St. John's wort and rifampicin, antacids that elevate gastric pH, and inhibitors of hOCT1.[99,103–105] These pharmacokinetic interactions are clinically relevant because clinicians have to alter treatment and dosage depending on coadministered drugs that may be effecting the absorption, distribution, or elimination of the therapy drug. Also, pharmacokinetic interactions may increase imatinib exposure to cells, and pharmacodynamic interactions may be a consequence of this increased exposure.

Antibiotic medications are often administered to patients with cancer while receiving anticancer treatments including imatinib. These patients are vulnerable to pharmacokinetic or pharmacodynamic interactions. For example, both imatinib and sulfamethoxazole are known competitive inhibitors of CYP2C9, a hepatic enzyme known for metabolizing many drugs, and this may affect the concentration or the effect of SMX/TMP or imatinib in patients.[106,107] Other anti-infective agents have been known to alter imatinib exposure in patients including clarithromycin, erythromycin, ciprofloxacin, levoflaxicin, and many others mostly due to interactions with CYP3A4, CYP2C9, hOCT1, or Pgp.[99]

Renal impairment can be another factor that contributes to the change in the pharmacokinetics of imatinib. Antibiotics are often responsible for causing acute renal injury (AKI), with 18% of hospitalized patients developing AKI during their stay while being treated for an infectious disease.[108] When assessing patients with cancer that had varying degree of renal impairment, the mean imatinib exposure increased 1.5- to 2-fold

compared to patients with normal renal function.[109] This can lead to adverse drug reactions with imatinib and it is important for clinicians to continually monitor this anti-cancer therapy when these reactions occur.

Despite the many pharmacokinetic interactions with imatinib described, the clinical relevance of many pharmacodynamic interactions with imatinib is unknown in adult Ph^+ leukemia patients.[110] Identifying the effects of possible interactions between antibiotic medications commonly taken by patients with Ph^+ leukemia will help clinicians optimize TKI therapy and help improve patient care.

Assay Development

Analytical assays are important methods required to understand and measure properties of a target, such as cells, drugs, or other biochemical substances. They can be used to identify drug interactions that may results in synergy or antagonism. Cell-based assays can measure the function of the target in the context of the cell, including cell viability and proliferation. Constructing an assay that is specific, sensitive, and robust is crucial in many areas of research, drug development, and clinical use.[111] Assay development requires identifying the purpose of the analytical method, determining the method and the necessary steps required, establishing specification limits, performing a risk assessment, and validating the measurement performance.[112] Some key considerations in assay development include robustness, reliability, practicality, cost, and automation.[113]

Cell-based assays that test for cell viability are often used to screen different compounds and their effect on cell proliferation. There are a number of different assay

methods use to test for cell viability including tetrazolium reduction, resazurin reduction, protease markers, and ATP detection.[114] Each cell viability assay has associated advantages and disadvantages. ATP detection is the easiest and fastest to perform, the most sensitive, and has the least amount of interference, while the resazurin and tetrazolium compounds are less expensive alternatives.[114] Cell-based assays aim to mimic the *in vivo* cellular environment, and these types of assays are becoming a common method to test variations in cellular conditions.

Purpose and Goals of Thesis Project

The main purpose of this thesis project is to develop high-throughput screening technology to test potential anticancer therapy drugs and frequently concomitantly administered drugs and to determine whether the antibiotics bactrim, gentamicin, and ceftazidime, which are commonly used for prevention and treatment of bacterial infections in leukemia patients, can interfere with the antileukemia therapy imatinib in patients with Ph^+ leukemia. This will help clinicians optimize TKI therapy and improve patient care. The goals of this project is to develop a protocol for *in vitro* testing that can be used in the future to determine other TKI-drug interactions:

- Establish optimal cell proliferation conditions for a high-throughput assay.
- Establish inhibitory concentrations of TKI's, imatinib and dasatinib on Ph^+ cell line K562 proliferation.
- Determine the effects of the commonly used antibiotic medications bactrim, gentamicin, and ceftazidime on cell growth of the Ph^+ cell line K562 treated with imatinib.

MATERIALS AND METHODS

Reagents

Imatinib mesylate, dasatinib, gentamicin sulfate (≥590 μg gentamicin base per mg), sulfamethoxazole, trimethoprim, and ceftazidime were purchased from Sigma-Aldrich (St. Louis, MO) and stored at 4°C protected from light. Prior to experiments, imatinib mesylate and dasatinib were dissolved in DMSO (Thermo Fisher Scientific, Waltham, MA) while gentamicin sulfate, sulfamethoxazole, trimethoprim, and ceftazidime were dissolved in distilled H_2O with 0.3% Tween 20 (solutions based on recommendations by Tecan technical support for optimized instrument dispensing). RPMI-1640 cell culture media, fetal bovine serum, penicillin-streptomycin-amphotericin, and L-glutamine were purchased from Thermo Fisher Scientific.

Cell Culture

K562 is a nonadherent cell line derived from a patient with Philadelphia chromosome positive chronic myelogenous leukemia.[115] K562 cells were obtained from American Type Culture Collection (Manassas, VA) and maintained according to the recommended protocol. Briefly, cells were incubated in complete media comprised of RPMI-1640 media supplemented with 10% fetal bovine serum, penicillin-streptomycin-amphotericin [10μg/mL], and L-glutamine [2mM] at 37°C and 5.5% CO_2. Cells were passed when reached 80% confluency. The complete media was stored at 4°C and

warmed to 37°C when added to the culture. K562 cells were collected at the first, second, and third passage for freezing. Frozen stocks of K562 cells were stored in liquid nitrogen suspended in a 10% DMSO and complete media solution.

Cell Seeding Density Determination

A stock concentration of K562 cells (8 x 10^4 cells/mL or 2,000 cells/25 μL) was used to make twofold serial dilutions of 25 μL/well in complete RPMI media into a 384-well plate. These dilutions were done in triplicates. Final cell concentrations ranged from 2,000 K562 cells/25 μL to 31 K562 cells/25 μL. Each sample was plated in triplicate. Cell proliferation was measured at time 0 hours, 24 hours, 48 hours, and 72 hours. Number of cells present in each well was counted in a hemocytometer under a microscope using trypan blue exclusion of dead cells. The trypan blue dye exclusion protocol used was developed from *Current Protocols in Immunology*, 2015.[116] Living cells have intact cell membranes, which exclude certain dyes, such as trypan blue, from entering into the cell, whereas dead cells do not. Therefore, cells positive for trypan blue dye have compromised membrane integrity, indicating loss of viability. The procedure outline an equal amount of cell suspension mixed with 0.4% trypan blue. A drop (10 μL) was applied to a hemacytometer, and the unstained (viable) cells were counted under a light microscope. The percentage of viable cells was then calculated. This experiment was repeated twice in triplicate. This assay was performed to measure K562 cell proliferation within a 72-hour period and to determine a reliable time of drug administration (when the cells are in exponential phase and growing freely).

CellTiter-Glo® Luminescent Cell Viability Assay

After optimal cell concentrations were determined, the CellTiter-Glo® Luminescent Cell Viability Assay was used to determine the number of viable cells present in each well. The CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI) was performed according to the manufacturer recommendations. [117] The assay is based on the measurement of the ATP present, which is proportional to the number of metabolically active cells present in culture. Mono-oxygenation of luciferin (present in the CellTiter-Glo® Reagent) is catalyzed by luciferase (also present in the the CellTiter-Glo® Reagent) in the presence of Mg^{2+}, ATP, and molecular oxygen. Briefly, the CellTiter-Glo® Buffer (100 mL) was added to the lyophilized CellTiter-Glo® Substrate at room temperature (23°C) to create the CellTiter-Glo® Reagent. This Reagent was added in equal volume to the medium containing cells present in each well (25 µL). The plate was incubated at room temperature for 20 minutes protected from light. Luminescence was recorded using the Synergy™ Neo2 Multi-Mode Microplate Reader (BioTek, Winooski, VT).

K562 cells at concentration 3.36 x 10^6 cells/mL or 84,000 cells/25 µL in complete media were plated into a 384-well plate and serial twofold dilutions were made with four replicates via pipette. The dilutions ranged from 84,000 K562 cells/25 µL to 328 K562 cells/25 µL. A larger range of cell concentrations were used compared to the previous experiment because this wider range of cells accounted for the proliferation of cells after the culture period. Plates with cells were wrapped in water moistened paper towels (based on recommendation from the Drug Discovery Core Director to prevent evaporation) and kept at 37°C in 5.5% CO_2. After 1 hour, resting cell viability was measured using the

CellTiter-Glo® Luminescent Cell Viability Assay.

Imatinib and Dasatinib Inhibitory Concentration Determination

K562 cells in complete media were dispensed into a 384-well plate from a stock concentration of 8 x 10^4 cells/mL or 2,000 cells/25 μL based on experimental data using the Freedom EVO® liquid handling workstation (Tecan, Männedorf, Switzerland, Figure 1A). Immediately following cell dispensing, lids were placed on the 384-well plate, wrapped with a water moistened paper towel, and stored at 37°C in 5.5% CO_2. After incubation for 24 hours, cells were exposed to a tyrosine kinase inhibitor (imatinib mesylate or dasatinib). TKI's were dispensed using the HP D300 Digital Dispenser (Hewlett-Packard, Palo Alto, CA, Figure 1C). Imatinib mesylate was diluted to a top stock concentration of 10 mM and dasatinib was diluted to a top stock concentration of 5 mM. Imatinib concentrations that were tested ranged from 30 μM to 3.17 nM with ½ log and 1:1.3 distributions. Dasatinib concentrations that were tested ranged from 10 μM to 0.5 pM with ½ log and ¼ log distributions. Percent DMSO was kept constant across the wells. Drug dispensing protocols were established to reduce the occurrence of edge effect. Figure 1B shows the protocol for imatinib distribution into the 384-well plate using the program for the HPD300 Digital Dispenser. After the drugs were dispensed, plates were covered with a water moistened paper towel and kept at 37°C in 5.5% CO_2 for 48 hours prior to performing cell proliferation assays. Seventy-two hours after the start of the experiment, cell proliferation was determined using the CellTiter-Glo® Luminescent Cell Viability Assay and a Synergy™ Neo2 Multi-Mode Microplate Reader (Figure 1D). Nonlinear regression analysis was performed using Prism 7 (Graphpad, La

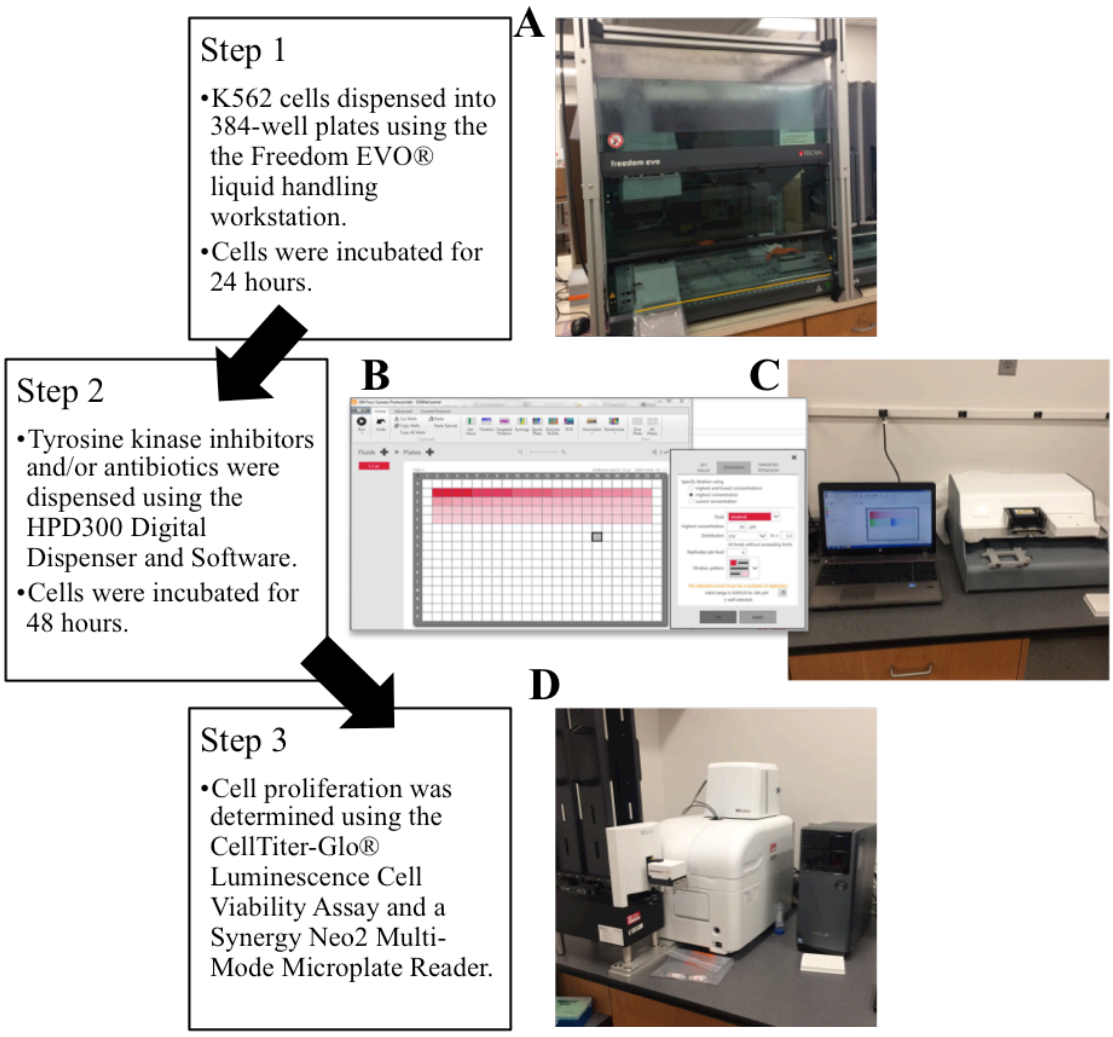

Figure 1. Diagram depicting the protocol used to determine TKI inhibitory concentrations and antibiotic perturbation on TKI efficacy. The following pictures were taken at the drug Discovery Facility at the University of Utah: A) Freedom EVO® liquid handling workstation. B) HPD300 Digital Dispenser Software with example dispensing protocol. C) HPD300 Digital Dispenser. D) Synergy Neo2 Multi-Mode Microplate Reader.

Jolla, CA) to determine inhibitory concentrations (IC_{10}, IC_{50} (half maximal), and IC_{90}) of imatinib and dasatinib based on residual viable cells across three independent experiments.

Assessing Potential Antibiotic Perturbation of TKI Efficacy

Once the IC_{10}, IC_{50}, and IC_{90} for imatinib were determined, these concentrations were tested with different antibiotics (gentamicin, sulfamethoxazole, trimethoprim, and ceftazidime) at concentrations reflecting clinically relevant exposures.[118–120]

K562 cells were dispensed into five 384-well plates using the same protocol as for the inhibitory concentration experiment (see above). After 24 hours, TKI plus antibiotic agent were dispensed into the plates using the HP D300 Digital Dispenser to reflect a pure co-administration. Imatinib mesylate was diluted to the concentration 5 mM using DMSO. Gentamicin sulfate, ceftazidime, sulfamethoxazole, and trimethoprim were diluted to 10 mM with distilled H_2O plus 0.3% Tween 20. Gentamicin concentrations that were tested ranged from 200 μM to 0.5 μM. Ceftazidime concentrations ranged from 300 μM to 10 μM, sulfamethoxazole ranged from 1 mM to 10 μM, and trimethoprim ranged from 43.5 μM to 0.43 μM. Another plate containing both trimethoprim and sulfamethoxazole was distributed 1:23, respectively, and the concentration ranges were the same as the individual plate concentrations described above for trimethoprim and sulfamethoxazole.

Imatinib was dispensed across each of these antibiotic's concentration ranges at 0.8 μM (IC_{10}), 2.2 μM (IC_{50}), and 5.9 μM(IC_{90}). The wells were normalized to volume DMSO and distilled H_2O plus 0.3% Tween 20. Control wells included K562 cells

incubated with antibiotic only, imatinib at IC_{10}, IC_{50}, and IC_{90}, solvent only (DMSO and distilled H_2O plus 0.3% Tween 20), and media. The cells were cultured for 48 hours using the same protocol as the previous experiments. Seventy-two hours after dispensing the cells, the cell proliferation was measured using the CellTiter-Glo® Luminescent Cell Viability Assay and a Synergy™ Neo2 Multi-Mode Microplate Reader.

A perturbation index was calculated for each antibiotic. The perturbation index was determined as the relative luminescence (cell viability) of the antibiotic medication plus imatinib at the inhibitory concentration over the relative luminescence (cell viability) of imatinib alone. The antibiotic was then judged as antagonistic (>1), synergistic (<1), or having no effect on the efficacy of imatinib (=1).

Statistical Analysis

The statistical significance of differences observed between experimental and controls groups were determined using the one-way analysis of variance (ANOVA) test. The post-hoc Dunnett's test was used to compare each experimental value with an imatinib-only control value. A linear trend test was done to determine if the relative luminescence (cell viability) increased or decreased systematically as the antibiotic concentrations ranged from low to high across the imatinib inhibitory concentrations. Differences were considered statistically significant when p values were <0.05.

RESULTS

Determination of Reliable Cell Concentration of K562 Cells for Proliferation Assay

A reliable concentration of K562 cells in 25 µL in a 384-well plate was first established to use in a future high-throughput assay. Two thousand K562 cells in 25 µL of complete media per well were plated in triplicates and twofold serial dilutions were performed. The dilutions ranged from 2,000 K562 cells/25 µL per well to 31 K562 cells/25 µL per well ($8x10^4$ cells/mL to 1,240 cells/mL). Cell proliferation was determined at 0 hours, 24 hours, 48 hours, and 72 hours. It has been reported that the optimal concentration of K562 cells for inhibition of proliferation assay is 2-5 x 10^4 cells/mL.[121,122]

Figure 2 shows the mean number of live cells in 25 µL after a given amount of time. Viable cells present in cell suspension were measured using the trypan blue dye exclusion test. Each series varies based on the number of cells that were counted at the time-point zero hours. The error bars depict the 95% confidence interval of three replicates for each cell concentration and time point. When the highest concentration of cells (2,000 cells/25 µL or 8 x 10^4 cells/mL) was plated, cell number expanded ~3.6-fold after 72 hours compared to the starting concentration. The cells with the next highest starting concentration (625 cells/25 µL or 2.4 x 10^4 cells/mL) expanded 7.1 times by 72 hours compared to the starting concentration. The starting concentration of cells of

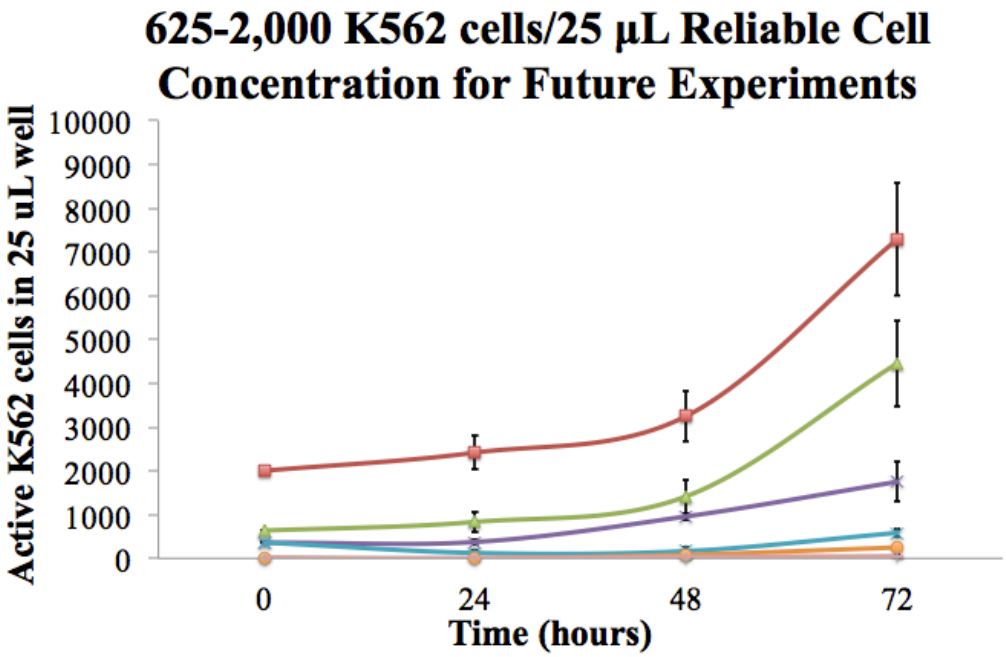

Figure 2. 625 – 2,000 K562 cells/25 µL reliable cell concentration for future experiments. Each series varies based on the number of cells that were counted at the time-point zero hours (K562 cells/25 µL well): red - 2000, green - 625, purple - 375, blue - 188, orange - 125, pink - 62. The error bars depict 95% confidence interval of three replicates for each cell concentration and time point.

375 cells/25 µL (1.5 cells/mL) also significantly proliferated (4.7 times), while the remaining concentrations did not demonstrate any significant proliferation (<2 times).

After 72 hours, the top two concentrations (2,000 cells/25 µL and 625 cells/25 µL) are in log phase of cell growth and haven't reached stationary phase (Figure 2). The lower starting concentrations continue to be in a lag phase after 72 hours. Therefore, lower plating densities are not optimal for assessment of cellular function and proliferation. The results suggest that a density of 2.5-8 x 10^4 cells/mL of K562 cells is a reliable concentration of cells for future experiments.

Association Between Luminescent Signal and Cell Number Using the CellTiter-Glo® Luminescent Cell Viability Assay

The relationship between luminescence measured with the CellTiter-Glo® Luminescent Cell Viability Assay and the number of K562 cells in culture was determined by making serial twofold dilutions in a 384-well plate in complete media. The dilutions ranged from 84,000 K562 cells/25 µL per well to 328 K562 cells/25 µL per well. The luminescence assay was recorded using the CellTiter-Glo® Assay and the results are presented in Figure 3.

Figure 3 depicts the relationship between luminescence values from the CellTiter-Glo® Assay and the K562 cell count per well. Luminescence values ranged from 3.9×10^6 to 4.2×10^4 relative luminescence units (RLU). The error bars depict the 95% confidence interval of four replicates (n=2) for each cell concentration with an R^2 of 0.956 (y=46.85x+277928). Concentrations below 21,000 cells/25 µL per well demonstrated an R^2 of 0.99723 (y=98.515x+24028). The luminescent signal from ~328 cells/25 µL per well (the lowest concentration of cells measured) is 168x the background signal from medium without cells, indicating that there is not a significant deviance from linearity between the luminescent signal and the number of cells from 0 to 8.4×10^5 cells/mL or 21,000 cells/25 µL. According to Figure 2, a starting concentration of 2,000 K562 cells/25 µL will proliferate to ~7,000 cells/25 µL after 72 hours of culture, generating a luminescence signal at $\sim 8 \times 10^5$, indicating 2,000 K562 cells/25 µL is an acceptable starting concentration to use for further experimentation.

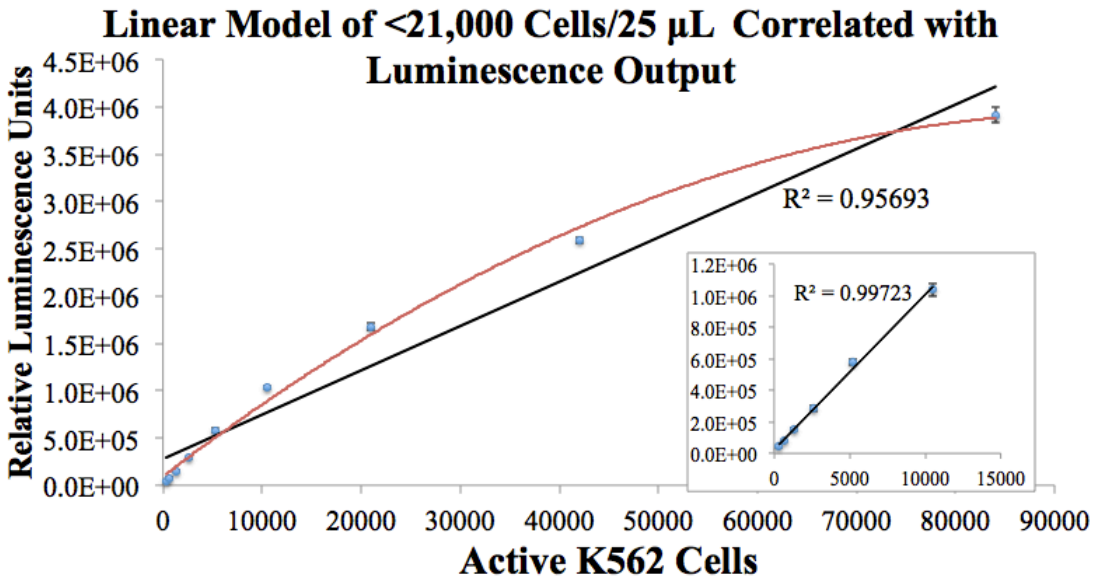

Figure 3. Linear model of <21,000 cells/25 µL correlated with luminescence output. The black line represents the linear model fit to the data points and the red line represents the polynomial trendline. The error bars depict the 95% confidence interval.

Imatinib and Dasatinib IC_{50} Determination

TKI's are used as the primary treatments for Philadelphia chromosome positive leukemia because they inhibit the bcr-abl tyrosine kinase, which leads to a reduction in kinase activity.[48] Table 1 outlines the mechanisms of action for imatinib and dasatinib. The half maximal inhibitory concentrations (IC_{50}) of imatinib and dasatinib with K562 cells were determined by dispensing the TKI's (Imatinib: 30 µM to 3.17 nM; Dasatinib: 10 µM to 0.5 pM). Cell viability was assessed by measuring luminescence using the cell viability assay.

The concentration ranges of the first set of experiments with imatinib and dasatinib proved to be insufficient to accurately establish upper and lower bounds to assess IC_{50} (Figure 4A and 4B). The original ranges were established at 10 µM to 0.1 nM for imatinib and 10 µM to 0.1 nM for dasatinib. The initial results for imatinib depicted

Table 1 - Mechanisms of Action for Imatinib and Dasatinib	
TKI	Mechanism of Action
Imatinib	Targets the well-conserved nucleotide-binding pocket of Abl close to the ATP binding site; binds to the inactive conformation of the activation loop of Abl.[48]
Dasatinib	Binds ATP-binding site in the active and inactive conformation of Abl.[59]

only the maximal percent live cells; this indicated that higher concentrations of imatinib needed to be examined (Figure 4A). The minimum percent live cells were only seen in the initial results of dasatinib, which indicated that lower concentrations of dasatinib should be tested (Figure 4B). After adjusting the concentration ranges (imatinib: 30 μM to 3.17 nM; dasatinib: 10 μM to 0.5 pM), a better defined sigmoidal curve was produced and these data were used to calculate the inhibitory concentrations of both imatinib and dasatinib on K562 cells. The three independent experiments with four replicate wells with the adjusted concentration ranges are seen in Figure 4C and 4D.

The IC_{50} of imatinib and dasatinib were estimated by using nonlinear regression analysis with Prism 7 Software. Imatinib's IC_{10} (95% CI, p-value) was estimated to be 0.8 μM (0.020, <0.001), the IC_{50} is 2.2 μM (0.009, <0.001), and the IC_{90} is 5.9 μM (0.019, 0.6991). Dasatinib's IC_{10} is 0.07 nM (0.032, 0.0093), the IC_{50} is 0.25 nM (0.015, <0.001), and the IC_{90} is 0.95 nM (0.031, 0.0078). Figure 4C and 4D depicts the relationship between the relative luminescence values and imatinib and dasatinib concentrations, which were used to calculate the IC_{50} of each. The error bars for each graph depict the 95% confidence interval.

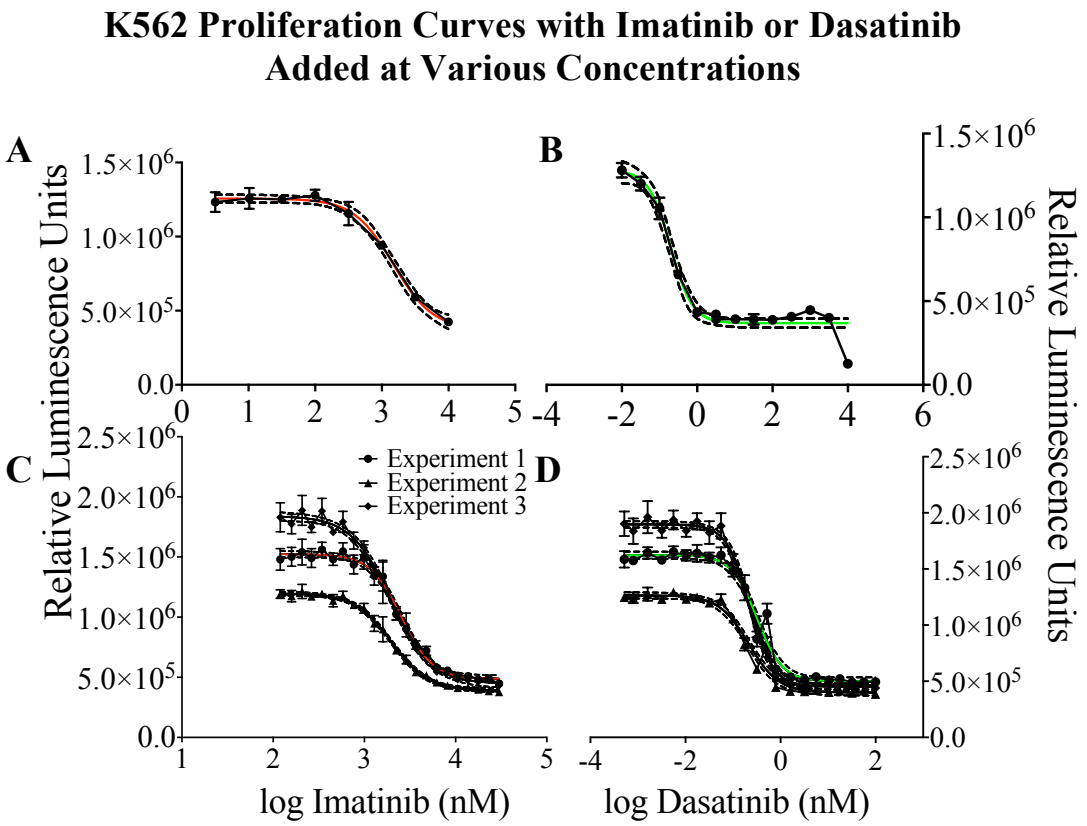

Figure 4. K562 proliferation curves with imatinib or dasatinib added at various concentrations. Cell proliferation was measured after 72 hours of culture with the CellTiter-Glo Assay. A) Original Range for Imatinib (10 μM to 0.1 nM). B) Original range for Dasatinib (10 μM to 0.1 nM). C) Adjusted concentrations for Imatinib (30 μM to 3.17 nM), producing a sigmoidal curve for the three trials performed. D) Adjusted concentrations for Dasatinib (10 μM to 0.5 pM), producing sigmoidal curve for the three trials performed. Error bars for each graph depict the 95% confidence interval.

Figure 5 depicts the residual plots from the three trials with the adjusted concentrations for imatinib and dasatinib. The residual plot for imatinib (Figure 5A) shows the data are evenly distributed around the regression line. The lower imatinib concentrations display increased variance heterogeneity compared to the higher concentrations. This can also be noted in Figure 4C: there is more variability in the lower imatinib concentrations between the three experiments compared to the higher concentrations.

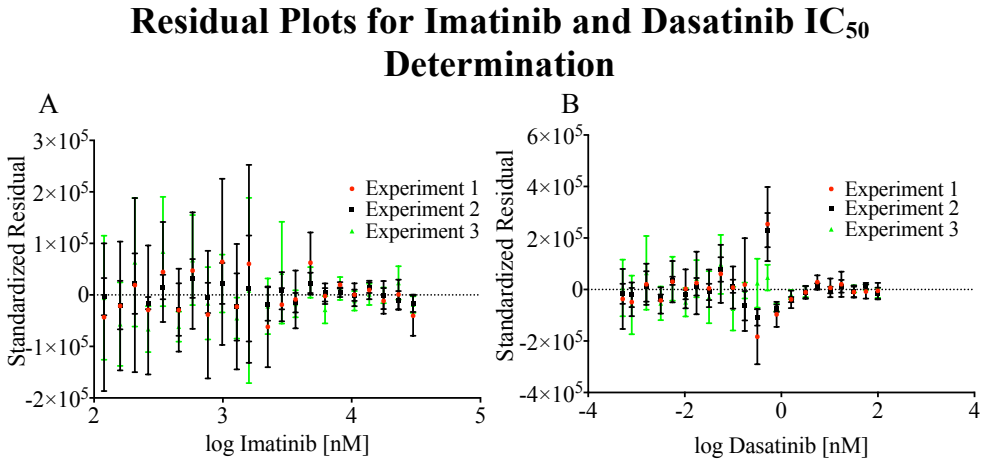

Figure 5. Residual plots for imatinib and dasatinib IC_{50} determination. The errors bars depict the 95% confidence interval.

The dasatinib residual plot also shows a consistent distribution around the regression line (Figure 5B). However, during experiment 1 and 2, a consistent deviation was seen between the dasatinib concentrations 0.52 nM and 0.32 nM (log -0.3 and log -0.49) shown in Figure 4D and 5B, indicated by an elevation in relative luminescence units beyond flanking dasatinib concentrations. Experimentally, a change in top stock concentrations occurred between these two dasatinib concentrations from 1 mM to 1 µM because the instrument cannot accurately dispense a volume below 20 pL. After experiments 1 and 2, the stock concentrations of dasatinib were changed from 1 mM and 1 µM to 20 µM and 100 nM. This change in stock concentration altered the smallest amount of volume that was dispensed into the wells while keeping the final concentration the same. This change in protocol in experiment 3 caused those two concentrations (0.52 nM and 0.32 nM) to become significantly closer to the regression line.

Solvents Used in TKI Efficacy Assay

Imatinib mesylate and dasatinib were dissolved in DMSO. However, DMSO has shown to be toxic to human cell lines at concentrations as low as 2-4% and it was important to determine the concentration of DMSO that was nontoxic to K562 cells.[123] Figure 6 depicts theK562 cell proliferation curve that was created when different amounts of DMSO were administered to K562 cells at the starting concentration 2,000 K562 cells/25 μL. Based on these data, the amount of DMSO is imperative to getting consistent results with K562 cells and must be kept below 0.5% to not get DMSO-biased results. In order to keep DMSO concentrations low, a higher stock concentration of Imatinib (5 mM) was created to prevent further issues with DMSO. DMSO volume was kept constant for all wells.

All of the antibiotics were dissolved in distilled H_2O with 0.3% Tween 20. Tween 20 is also known to be toxic to cells at certain concentrations.[124] The results showed that concentrations of <5% of the water/tween 20 solution did not have detective influence on the viability of K562 cells; however, >10% of the water/tween 20 solution did show a significant impact on the K562 cells viability. Figure 7 depicts the relationship between increasing water/tween 20 solution concentration and K562 cells viability (measured in relative luminescence units). These data indicated that keeping the concentration of Tween 20 low was important to maintain unbiased results. Higher top stock concentrations of antibiotics were used to prevent Tween 20 from affecting K562 cell proliferation.

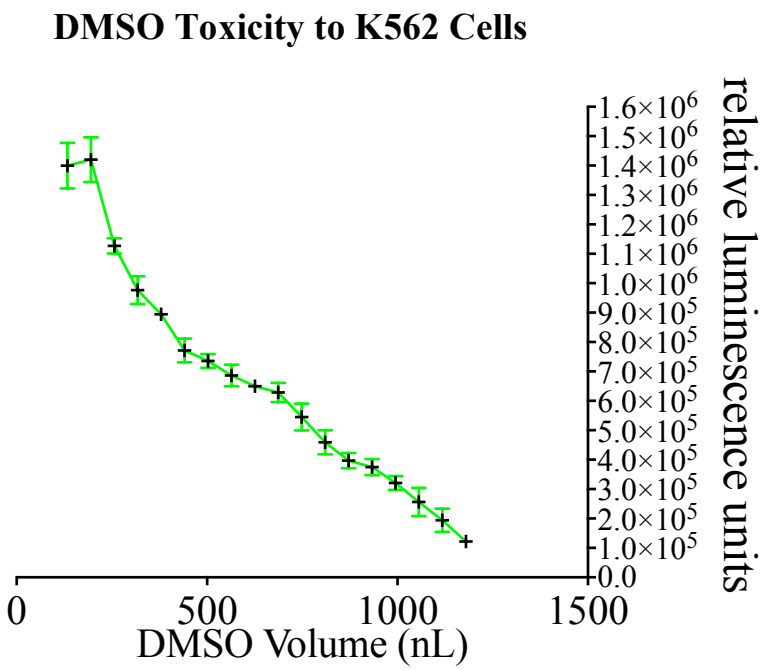

Figure 6. DMSO toxicity to K562 cells. Increasing volume of DMSO shows a decrease in relative luminescence units, indicating a decrease in percentage of live K562 cells. The error bars depict the 95% confidence interval.

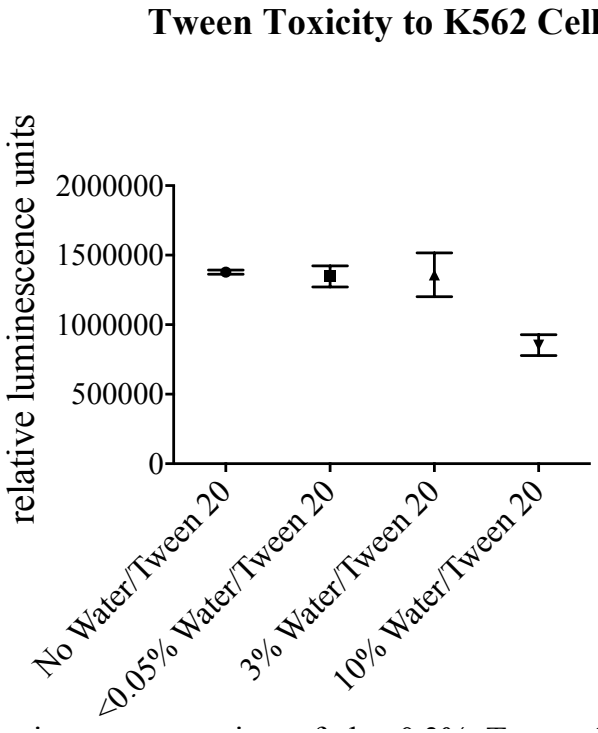

Figure 7. Tween toxicity to K562 cells. Increasing concentration of the 0.3% Tween 20 H_20 solution (Water/Tween 20) shows toxicity to K562 cells. The error bars depict the 95% confidence interval.

Determination of Imatinib Efficacy after Co-incubation with Antibiotics

Imatinib efficacy after the addition of antibiotics was determined by culturing the TKI at the IC_{10}, IC_{50}, and IC_{90} determined in the previous experiments and an antibiotic at ten-times above and below clinically relevant concentrations with K562 cells. Cell viability was determined 48 hours after dispensing the drugs.

Figure 8A-J depicts the results of the experiments that examine imatinib's ability to suppress K562 proliferation after co-incubation with antibiotics. Figures 8A, C, E, G, and I compare the concentration of the antibiotics with the cell viability (measured in relative luminescence units) when Imatinib is added at IC_{10}, IC_{50}, and IC_{90}. Figures 8B, D, F, H, and J compare the perturbation ratio of luminescence with imatinib at IC_{10}, IC_{50}, and IC_{90} co-incubated with the antibiotics over the luminescence of imatinib alone (at IC_{10}, IC_{50}, and IC_{90}, respectively). A perturbation ratio equal to one indicates that the antibiotic did not alter the efficacy of imatinib relative to imatinib treatment alone. A ratio greater than one suggests the antibiotic is antagonistic and reduces the ability of imatinib to decrease K562 cell proliferation. A ratio less than one designates the antibiotic to be synergistic, which causes an even greater reduction in K562 cell proliferation.

Gentamicin and ceftazidime appear to have a perturbation ratio slightly lower than one for all imatinib concentrations (Figure 8B and D), which indicates they have a slight antagonistic effect on imatinib, whereas sulfamethoxazole and trimethoprim are fairly equal to one and have no effect (Figure 8F, H and J). Statistically, gentamicin and ceftazidime show to be significant through the ANOVA post-hoc linear trend test, which indicates that the slopes in Figure 8A and C have a significant slope not equal to zero

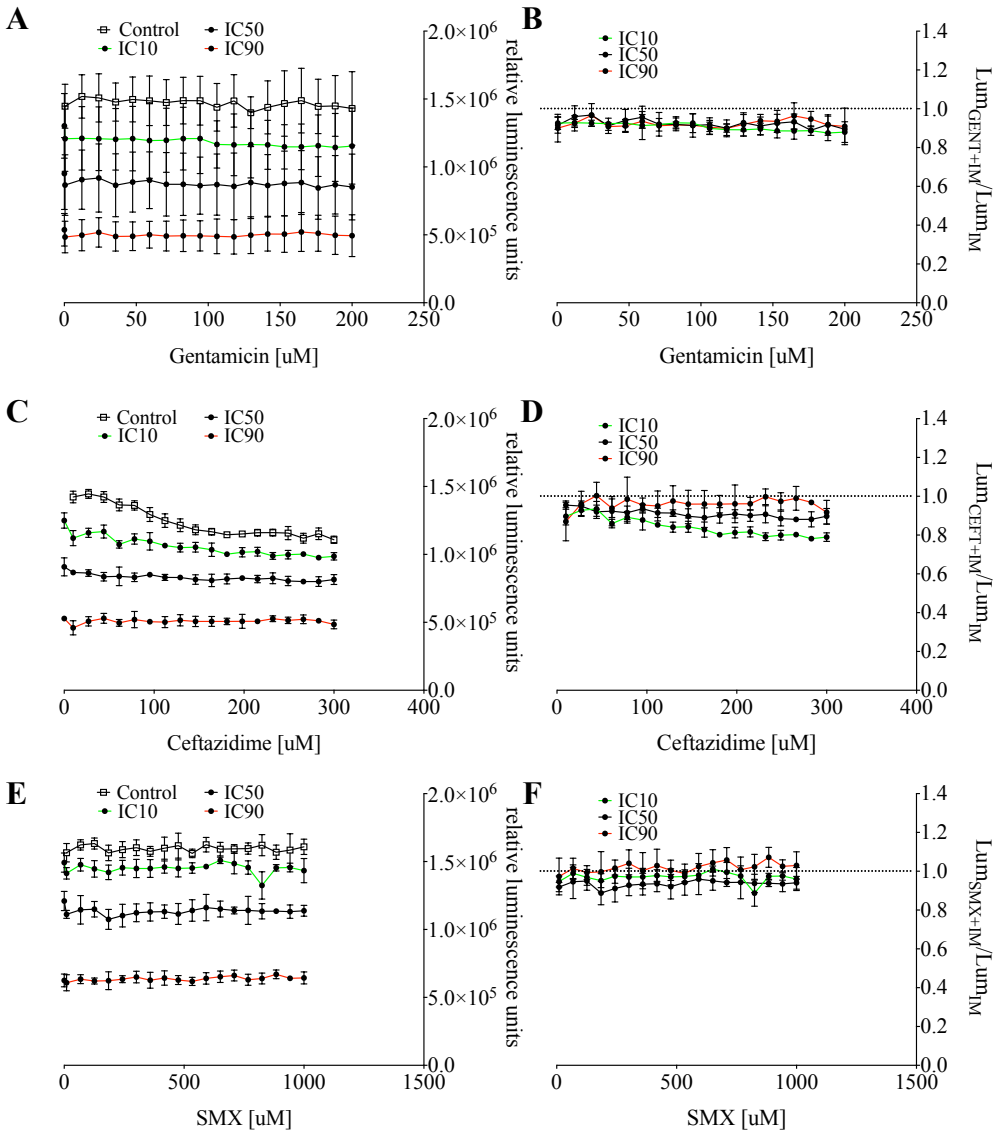

Figure 8. K562 cell proliferation with co-incubation of imatinib and antibiotics and perturbation ratio. Cell proliferation was measured in luminescence units after 72 hours of culture. Imatinib concentrations were the previously determined IC_{10}, IC_{50}, and IC_{90}. Imatinib was co-incubated with A) gentamicin, C) ceftazidime, E) sulfamethoxazole, G) trimethoprim, and I) trimethoprim and sulfamethoxazole distributed 1:23, respectively. Controls contain no imatinib, only antibiotics. The perturbation ratio of luminescence with imatinib (IC_{10}, IC_{50}, and IC_{90}) and antibiotics over the luminescence with only imatinib (IC_{10}, IC_{50}, and IC_{90}) compared to the concentrations of antibiotics is seen in the following figures with antibiotics: B) gentamicin, D) ceftazidime, F) sulfamethoxazole, H) trimethoprim, and J) trimethoprim and sulfamethoxazole distributed 1:23, respectively. The error bars depict the 95% confidence interval.

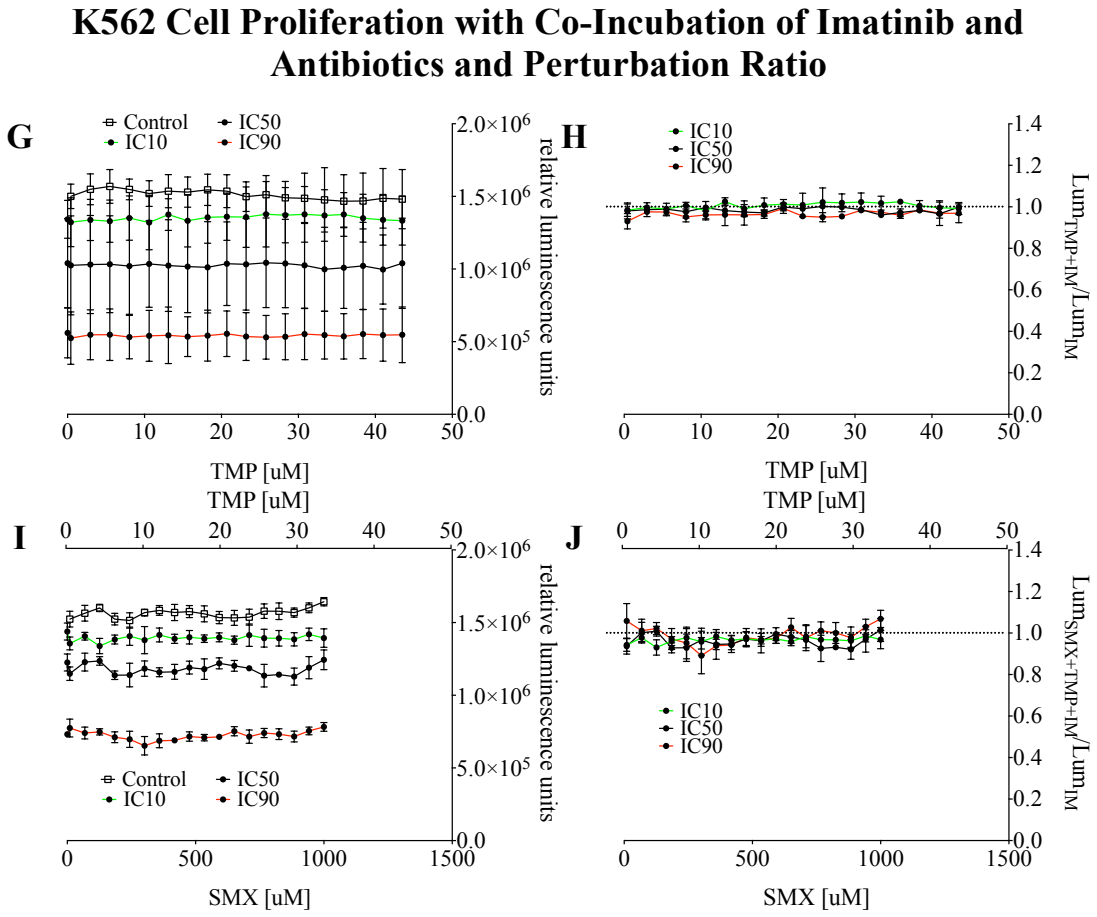

Figure 8 continued.

(Table 2; P=0.003 and <0.0001, respectively). However, they were not statistically significant through the ANOVA or Dunnett test (Table 2). This indicates that gentamicin and ceftazidime have a significant effect on the proliferation of K562 cells, but the concentration of imatinib does not alter the effect. Sulfamethoxazole, trimethoprim or the sulfamethoxazole:trimethoprim ratio were not seen significant in any of the three tests (Table 2).

The controls for these tests are shown in Figure 9. The controls relative luminescence values vary between experiments, but they all vary in the same direction. This indicates that the results are precise, but not as accurate. However, the accuracy is not as important as the precision for obtaining these results because a ratio is used to determine the overall effect the antibiotics have on the efficacy of imatinib.

Table 2 – ANOVA and Post-Hoc Test Results on Co-Incubation of Imatinib and Antibiotics

	ANOVA	Dunnett Test	Linear Trend Test
	P value	Any Comparisons Significant (P<0.05)?	P value
Gentamicin	0.1615	No	0.003*
Ceftazidime	0.2667	No	<0.0001*
Sulfamethoxazole	0.2577	No	0.1248
Trimethoprim	0.4635	No	0.2737
1:23 Sulfamethoxazole: Trimethoprim	0.3469	No	0.2066

*Statistically significant

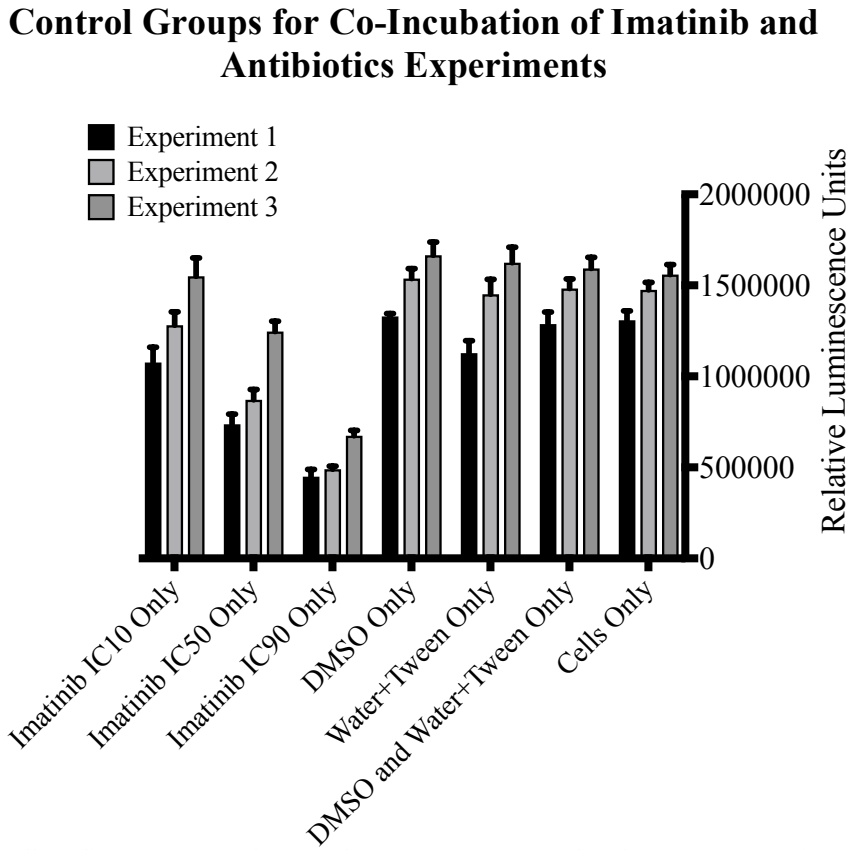

Figure 9. Control groups for co-incubation of imatinib and antibiotics experiments. Cell proliferation in each control group was measured in luminescence units after 72 hours of culture. The error bars depict the 95% confidence intervals.

DISCUSSION

The development of an assay that investigates the effect of tyrosine kinase inhibitors on the K562 cell line when incubated with other drugs is an important aspect of this thesis work because it can be used in future pharmaceutical research for tyrosine kinase inhibitors' ability to suppress K562 proliferation when administered with other drugs. Previous assays have been established to investigate K562 cell viability with TKI's and other drugs including other commercial luciferase luminescence cell viability assays, high performance liquid chromatography (HPLC), MTT cell proliferation assays, resazurin fluorescent cellular health indicators, and flow cytometry.[125–129] However, the protocol for this assay used innovative technology that has been proven to be robust and accurate.

Lee et al. used 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to measure K562 proliferation after incubation.[129] This protocol requires MTT to be incubated in the wells for 3-4 hours, removed through aspiration, and the absorbance of the formazan crystals (dissolved in DMSO) are measured.[129] This assay is highly sensitive; however, it requires many wash steps and cellular metabolic rate can interfere with the accuracy of the measurements, which can make this assay biased.[130] Similar to the protocol developed for this thesis work, the MTT assay is suitable for high-throughput screening with the assistance of technology.

The technology I used while developing this assay also allows for this protocol to

be used in high-throughput screening. The Freedom EVO® liquid handling workstation improved pipetting speed and reduced contamination, which improve the efficiency of these cell viability experiements.[131,132] The HP D300 Digital Dispenser has been tested against other cytotoxicity assays and determined to produce similar results as more traditional methods such as the pin tool.[133] This dispenser saves time by eliminating serial dilutions and requires low compound consumption.[133] This project utilized many of the features of the HP D300 Dispenser including randomized design and solvent normalization.

Other researchers, including Yamakawa et al., have used resazurin reduction assays like AlamarBlue®, which utilizes the reducing environment in viable cells to convert resazurin into resorufin, a compound that is highly fluorescent, to measure K562 cell proliferation.[127] Similar to the CellTiter-Glo® Luminescent Cell Viability Assay, few wash steps are involved. However, resazurin reduction assays are not cytotoxic like the CellTiter-Glo® Luminescent Cell Viability Assay, so follow-up assays can be performed on the same cells as the resazurin reduction assay. This feature is not necessary for this protocol that examines drug-drug interactions in the K562 cell line, but it may be a viable alternative to the CellTiter-Glo® Luminescent Cell Viability Assay if follow-up assays want to be performed in the future.

During the development of this assay, all TKI's were dissolved in DMSO, which proved to be a limitation to the assay. DMSO has been reported to have low toxicity to cells at concentrations <10%.[123] However, concentrations higher than 0.5% appeared to have a negative impact on the K562 cell proliferation during the development phase of this assay. Galvao et al. also reported similar results, that a low dose of DMSO (>1%) can

cause cellular toxicity through plasma membrane pore formation. Due to the DMSO toxicity, it was necessary for higher stock concentrations of TKI's to be used to reduce the amount of DMSO administered to cells.

The reliable K562 cell concentration was determined to be ~625-2,000 cells/25 μL, which was the same concentration that other researchers have derived to be a valid initial cell concentration.[134] After the assay was developed, it was validated by measuring cell proliferation with the addition of TKI's. The maximal inhibitory concentration of imatinib and dasatinib was determined by using the established protocol and the available instrumentation. This was an important step in developing an appropriate TKI-drug testing procedure because IC_{50} concentrations can vary based on the lab conditions, reagents, cell line mutations, and laboratory instrumentation. Published research studies have used a variety of IC_{50}'s for imatinib (0.15 - 4.4 μM) and dasatinib (0.35 - 1 nM) using the K562 cell line based on the conditions established through their protocol.[59,135–140] The combination of the IC_{10}, IC_{50}, and IC_{90} that were obtained from the assay development fits well within published values of imatinib.

However, the log IC_{10} and IC_{50} values that were produced for imatinib and dasatinib varied significantly. This may have been caused by the initial concentration of K562 cells that were cultured in the 384-well plate being slightly different. If the antibiotics were added across all of the concentrations of imatinib, there may have been a change in the effectiveness of imatinib. However, the three concentrations that were tested (IC_{10}, IC_{50}, and IC_{90}) were significantly different and they proved there was no change in imatinib efficacy across all three points.

After discovering appropriate TKI concentrations to test, imatinib was tested with

antibiotics commonly given to leukemia patients. Gentamicin, ceftazidime, trimethoprim, or sulfamethoxazole showed to not have a significant impact on the ability of imatinib to reduce K562 cell proliferation. Due to the time limit, this research was conducted with a low number of replicates for the imatinib-antibiotic drug combination. Ideally, 3-5 replicates would have produced a larger, less-biased data set to generalize the results for the population. However, this assay has the potential to conduct many more tests of imatinib-antibiotic interactions and other drug-drug interactions.

One limitation that should be noted is the control groups that were investigated were not similar throughout the different time-points. For example, the DMSO-only control varied from $1.3x10^6$ to $1.6x10^6$ relative luminescence units across all three experiments. This also may have been caused by the initial concentration of K562 cells plated being dissimilar. Although the values are all different, the ratios between them are similar. The first experiment has relatively less proliferation across all of the controls compared to the next two and the third experiment has the highest proliferation.

There was no antagonistic control among the control groups, which also proved to be a limitation to the experimental design. Without an antagonistic control, there is no independent variable that indicates an antagonistic effect to evaluate the protocol, reagents, and the equipment for experimental error. During future use of this protocol, drugs that indicate an antagonistic effect with imatinib should be further evaluated for use as a future antagonistic control in the experimental design. Similarly, drugs that are revealed to by synergistic should be evaluated for use as a synergistic control.

Drug-drug interactions have previously been studied in K562 cell lines, and both synergistic and antagonistic effects have been found. Tanespimycin, a derivate of the

antibiotic geldanamycin, was found to have synergistic activities with imatinib in AML patient cells by inhibiting Pgp-mediated imatinib efflux, thus increasing intracellular imatinib levels.[141] Another synergistic effect reported is that ABT-737, an analogue of an experimental orally active anticancer drug called navitoclax, enhances the effect of INNO-406, a second-generation BCR-ABL inhibitor named bafetinib.[125] Antagonistic activity against imatinib was found with the antipsychotic drug lithium chloride, where a combination of the two was less effective in decreasing cellular proliferation than imatinib alone.[142] The effectiveness of imatinib has also been known to be altered by St. John's wort, rifampicin, antacids that elevate gastric pH, and inhibitors of hOCT1.[99,103–105]

Although certain drugs have shown to have synergistic or antagonistic effects on TKI's, many drugs have been examined and determined to have no effect on TKI efficacy, similar to my findings with gentamicin, ceftazidime, and bactrim.[125] While *in vitro* studies cannot predict the *in vivo* microenvironment, like cytokine activity for example, that may contribute to the interactions that would occur with drugs, it is a good predictor of significant changes that can be caused by drug interactions. It is important for clinicians who are prescribing treatments regimens to leukemia patients to understand how concomitantly administered drugs can alter their effect. These physicians can now feel more confident when prescribing gentamicin, ceftazidime, or bactrim with imatinib to a patient because the antibiotics appear to have no effect on the efficacy of imatinib.

While no statistically significant effect was found between the antibiotics and imatinib under the testing conditions, a slight synergistic effect has been observed between gentamicin and imatinib. Potentially, under different conditions, this effect may

become significant and further testing may be necessary to further explore the relationship between gentamicin and imatinib.

One of the aims of this thesis work was to help develop an approach to testing leukemic cells' proliferation after the addition of one or more drugs. A high-throughput, semi-automated *in vitro* assay has been produced that can be used in the future to determine how other drugs that are commonly given to chronic myeloid leukemia patients affect TKI's. Some examples of drugs to be tested include acetaminophen, diphenhydramine, ondansetron, oxycodone, lorazepam, voriconazole, and morphine. This protocol can also be used to determine the IC_{50}'s of other TKI's (nilotinib and ponatinib) and how various pharmaceuticals impact their function.

In addition, other Ph^+ leukemic cell lines with different features from the K562 cell line can be tested to determine if variations in cell type, age/sex of patient, or disease status impact the effect medications have on TKI's to reduce cellular proliferation. The K562 cell line is erythrocytic, derived from a pleural effusion of a 53 year old female in CML blast crisis, and does not carry the classical Ph chromosome (but does have rearrangement of BCR and translocation of ABL).[143,144] One example of an alternative is the SUP-B15 cell line. The SUP-B15 is a B-cell precursor from a 9-year-old male with ALL during his second relapse.[143,145] Ph^+ ALL is much more common in children compared to adults, and therefore, studying a cell line that represents the younger ALL population would be informative for clinicians while administering supportive medications.

In this experiment, the TKI was dispensed at the same time as the antibiotics. However, in the clinic, antibiotics may be given a few days prior to the imatinib dose,

which may effect how the antibiotics interact with the cells and the TKI. To fully understand the clinical impacts of co-administration of TKI's and antibiotics, it would be necessary to pre-incubate the antibiotics *in vitro* before adding the TKI.

This approach to drug-drug interaction testing has the potential to be used in the identification of personalized treatments for patients with Ph^+ leukemia. Some of these therapies may be comprised of only two drugs, but can also be expanded to more than two.

CONCLUSION

In summary, using innovative technology and assay procedures, an effective, high-throughput *in vitro* assay has been developed that can be used to determine drug-drug interactions that would impact patients with Ph^+ leukemia using the K562 cell line. This assay was validated by testing K562 cell proliferation with various concentrations of the TKI's imatinib and dasatinib. Maximal inhibitory concentrations were determined and used to concomitantly test imatinib and commonly used antibiotics including gentamicin, ceftazidime, and bactrim (sulfamethoxazole and trimethoprim). While no statistically significant interactions were detected for any of the antibiotics, a slight synergistic effect has been observed for gentamicin and may be studied in the future. Finally, this new methodology can be used as a tool to predict drug efficacy and determine responses in Ph^+ leukemia patients that will lead to personalized treatment.

REFERENCES

1. Nowell PC. Discovery of the Philadelphia chromosome: a personal perspective. *J Clin Invest*. 2007;117(8):2033-2035. doi:10.1172/JCI31771.

2. Koretzky GA. The legacy of the Philadelphia chromosome. *J Clin Invest*. 2007;117(8):2030-2032. doi:10.1172/JCI33032.

3. Stam K, Heisterkamp N, Grosveld G, et al. Evidence of a new chimeric *bcr* /c- *abl* mRNA in patients with chronic myelocytic leukemia and the Philadelphia chromosome. *N Engl J Med*. 1985;313(23):1429-1433. doi:10.1056/NEJM198512053132301.

4. Kang Z-J, Liu Y-F, Xu L-Z, et al. The Philadelphia chromosome in leukemogenesis. *Chin J Cancer*. 2016;35:48. doi:10.1186/s40880-016-0108-0.

5. Score J, Calasanz MJ, Ottman O, et al. Analysis of genomic breakpoints in p190 and p210 BCR–ABL indicate distinct mechanisms of formation. *Leukemia*. 2010;24(10):1742-1750. doi:10.1038/leu.2010.174.

6. Clarkson B, Strife A, Wisniewski D, Lambek CL, Liu C. Chronic myelogenous leukemia as a paradigm of early cancer and possible curative strategies. *Leukemia*. 2003;17(7):1211-1262. doi:10.1038/sj.leu.2402912.

7. Dorey K, Engen JR, Kretzschmar J, et al. Phosphorylation and structure-based functional studies reveal a positive and a negative role for the activation loop of the c-Abl tyrosine kinase. *Oncogene*. 2001;20(56):8075-8084. doi:10.1038/sj.onc.1205017.

8. Zhang X, Subrahmanyam R, Wong R, Gross AW, Ren R. The NH(2)-terminal coiled-coil domain and tyrosine 177 play important roles in induction of a myeloproliferative disease in mice by Bcr-Abl. *Mol Cell Biol*. 2001;21(3):840-853. doi:10.1128/MCB.21.3.840-853.2001.

9. Harnois T, Constantin B, Rioux A, Grenioux E, Kitzis A, Bourmeyster N. Differential interaction and activation of Rho family GTPases by p210bcr-abl and p190bcr-abl. *Oncogene*. 2003;22(41):6445-6454. doi:10.1038/sj.onc.1206626.

10. Valent P. Targeting the JAK2-STAT5 pathway in CML. *Blood*. 2014;124(9):1386-

1388. doi:10.1182/blood-2014-07-585943.

11. Hoelbl A, Schuster C, Kovacic B, et al. Stat5 is indispensable for the maintenance of bcr/abl-positive leukaemia. *EMBO Mol Med*. 2010;2(3):98-110. doi:10.1002/emmm.201000062.

12. Steelman LS, Pohnert SC, Shelton JG, Franklin RA, Bertrand FE, McCubrey JA. JAK/STAT, Raf/MEK/ERK, PI3K/Akt and BCR-ABL in cell cycle progression and leukemogenesis. *Leukemia*. 2004;18(2):189-218. doi:10.1038/sj.leu.2403241.

13. Keeshan K, Cotter TG, McKenna SL. Bcr-Abl upregulates cytosolic p21 $^{WAF-1/CIP-1}$ by a phosphoinositide-3-kinase (PI3K)-independent pathway. *Br J Haematol*. 2003;123(1):34-44. doi:10.1046/j.1365-2141.2003.04538.x.

14. Kanakasetty GB, Kuntejowdahalli L, Thanky AH, et al. Predictive and prognostic implications of variant Philadelphia translocations in CML: experience from a tertiary oncology center in southern India. *Clin Lymphoma Myeloma Leuk*. 2016. doi:10.1016/j.clml.2016.09.007.

15. Chereda B, Melo J V. Natural course and biology of CML. *Ann Hematol*. 2015;94(S2):107-121. doi:10.1007/s00277-015-2325-z.

16. Höglund M, Sandin F, Simonsson B. Epidemiology of chronic myeloid leukaemia: an update. *Ann Hematol*. 2015;94(S2):241-247. doi:10.1007/s00277-015-2314-2.

17. Hijiya N, Schultz KR, Metzler M, Millot F, Suttorp M. Pediatric chronic myeloid leukemia is a unique disease that requires a different approach. *Blood*. 2016;127(4):392-399. doi:10.1182/blood-2015-06-648667.

18. Strom SS, Yamamura Y, Kantarijian HM, Cortes-Franco JE. Obesity, weight gain, and risk of chronic myeloid leukemia. *Cancer Epidemiol Biomarkers Prev*. 2009;18(5):1501-1506. doi:10.1158/1055-9965.EPI-09-0028.

19. What are the risk factors for chronic myeloid leukemia. American Cancer Society. https://www.cancer.org/cancer/chronic-myeloid-leukemia/causes-risks-prevention/risk-factors.html. Published 2016. Accessed February 15, 2017.

20. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2014 update on diagnosis, monitoring, and management. *Am J Hematol*. 2014;89(5):547-556. doi:10.1002/ajh.23691.

21. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood*. 2002;100(7).

22. Kantarjian HM, Keating MJ, Talpaz M, et al. Chronic myelogenous leukemia in blast crisis. *Am J Med*. 1987;83(3):445-454. doi:10.1016/0002-9343(87)90754-6.

23. Quintás-Cardama A, Cortes JE. Chronic myeloid leukemia: diagnosis and treatment. *Mayo Clin Proc*. 2006;81(7):973-988. doi:10.4065/81.7.973.

24. Kiss TL, Abdolell M, Jamal N, Minden MD, Lipton JH, Messner HA. Long-term medical outcomes and quality-of-life assessment of patients with chronic myeloid leukemia followed at least 10 years after allogeneic bone marrow transplantation. *J Clin Oncol*. 2002;20(9):2334-2343. doi:10.1200/JCO.2002.06.077.

25. Kantarjian H, O'Brien S, Jabbour E, et al. Improved survival in chronic myeloid leukemia since the introduction of imatinib therapy: a single-institution historical experience. *Blood*. 2012;119(9):1981-1987. doi:10.1182/blood-2011-08-358135.

26. Gambacorti-Passerini C, Antolini L, Mahon F-X, et al. Multicenter independent assessment of outcomes in chronic myeloid leukemia patients treated with imatinib. *JNCI J Natl Cancer Inst*. 2011;3(7):553-561. doi:10.1093/jnci/djr060.

27. Milojkovic D, Apperley J. Mechanisms of resistance to imatinib and second-generation tyrosine inhibitors in chronic myeloid leukemia. *Clin Cancer Res*. 2009;15(24).

28. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. *Lancet (London, England)*. 2013;381(9881):1943-1955. doi:10.1016/S0140-6736(12)62187-4.

29. Hunger SP, Mullighan CG. Redefining ALL classification: toward detecting high-risk ALL and implementing precision medicine. *Blood*. 2015;125(26):3977-3987. doi:10.1182/blood-2015-02-580043.

30. Bernt KM, Hunger SP. Current concepts in pediatric Philadelphia chromosome-positive acute lymphoblastic leukemia. *Front Oncol*. 2014;4:54. doi:10.3389/fonc.2014.00054.

31. Liu-Dumlao T, Kantarjian H, Thomas DA, O'Brien S, Ravandi F. Philadelphia-positive acute lymphoblastic leukemia: current treatment options. *Curr Oncol Rep*. 2012;14(5):387-394. doi:10.1007/s11912-012-0247-7.

32. PDQ adult treatment editorial Board. *Adult Acute Lymphoblastic Leukemia Treatment (PDQ®): Health Professional Version*.; 2002. http://www.ncbi.nlm.nih.gov/pubmed/26389171. Accessed March 9, 2017.

33. Signs and symptoms of acute lymphocytic leukemia. American Cancer Society. https://www.cancer.org/cancer/acute-lymphocytic-leukemia/detection-diagnosis-staging/signs-symptoms.html. Published 2016. Accessed March 9, 2017.

34. How is acute lymphocytic leukemia classified? American Cancer Society. https://www.cancer.org/cancer/acute-lymphocytic-leukemia/detection-diagnosis-

staging/how-classified.html. Published 2016. Accessed March 9, 2017.

35. Verrma SP, Dutta TK, Vinod K V, Dubashi B, Ariga KK. Philadelphia chromosome positive pre-T cell acute lymphoblastic leukemia: a rare case report and short review. *Indian J Hematol Blood Transfus*. 2014;30(Suppl 1):177-179. doi:10.1007/s12288-013-0314-8.

36. Koo HH. Philadelphia chromosome-positive acute lymphoblastic leukemia in childhood. *Korean J Pediatr*. 2011;54(3):106-110. doi:10.3345/kjp.2011.54.3.106.

37. Roberts KG, Gu Z, Payne-Turner D, et al. High frequency and poor outcome of Philadelphia chromosome-like acute lymphoblastic leukemia in adults. *J Clin Oncol*. 2017;35(4):394-401. doi:10.1200/JCO.2016.69.0073.

38. De Kouchkovsky I, Abdul-Hay M. Acute myeloid leukemia: a comprehensive review and 2016 update. *Blood Cancer J*. 2016;6(7):e441. doi:10.1038/bcj.2016.50.

39. Tasian SK, Pollard JA, Aplenc R. Molecular therapeutic approaches for pediatric acute myeloid leukemia. *Front Oncol*. 2014;4:55. doi:10.3389/fonc.2014.00055.

40. PDQ adult treatment editorial board. *Adult Acute Myeloid Leukemia Treatment (PDQ®): Patient Version*.; 2002. http://www.ncbi.nlm.nih.gov/pubmed/26389377. Accessed March 9, 2017.

41. Nacheva EP, Grace CD, Brazma D, et al. Does *BCR/ABL1* positive acute myeloid leukaemia exist? *Br J Haematol*. 2013;161(4):541-550. doi:10.1111/bjh.12301.

42. Soupir CP, Vergilio J-A, Cin PD, et al. Philadelphia chromosome–positive acute myeloid leukemia. *Am J Clin Pathol*. 2007;127(4):642-650. doi:10.1309/B4NVER1AJJ84CTUU.

43. Reboursiere E, Chantepie S, Gac A-C, Reman O. Rare but authentic Philadelphia-positive acute myeloblastic leukemia: two case reports and a literature review of characteristics, treatment and outcome. *Hematol Oncol Stem Cell Ther*. 2015;8(1):28-33. doi:10.1016/j.hemonc.2014.09.002.

44. Druker BJ. Perspectives on the development of imatinib and the future of cancer research. *Nat Med*. 2009;15(10):1149-1152. doi:10.1038/nm1009-1149.

45. Marshall J. Inner workings: tyrosine kinases, their discovery and impact. *Proc Natl Acad Sci U S A*. 2015;112(26):7886-7887. doi:10.1073/pnas.1510492112.

46. Al-Hadiya BMH, Bakheit AHH, Abd-Elgalil AA. Chapter six – imatinib mesylate. In: *Profiles of Drug Substances, Excipients and Related Methodology*. Vol 39. ; 2014:265-297. doi:10.1016/B978-0-12-800173-8.00006-4.

47. Reddy EP, Aggarwal AK. The ins and outs of bcr-abl inhibition. *Genes Cancer*. 2012;3(5-6):447-454. doi:10.1177/1947601912462126.

48. Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, Kuriyan J. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science (80-)*. 2000;289(5486).

49. Arora A, Scholar EM. Role of tyrosine kinase inhibitors in cancer therapy. *J Pharmacol Exp Ther*. 2005;315(3).

50. Druker BJ, Lydon NB. Lessons learned from the development of an abl tyrosine kinase inhibitor for chronic myelogenous leukemia. *J Clin Invest*. 2000;105(1):3-7. doi:10.1172/JCI9083.

51. le Coutre P, Mologni L, Cleris L, et al. In vivo eradication of human BCR/ABL-positive leukemia cells with an ABL kinase inhibitor. *J Natl Cancer Inst*. 1999;91(2):163-168. http://www.ncbi.nlm.nih.gov/pubmed/9923858. Accessed February 16, 2017.

52. Highlights of prescribing information. Novartis Pharmaceuticals Corporation. https://www.pharma.us.novartis.com/sites/www.pharma.us.novartis.com/files/gleevec_tabs.pdf. Published 2016. Accessed February 16, 2017.

53. Vapiwala N, Geiger G. Part one: introduction to small molecule tyrosine kinase inhibitors | OncoLink. OncoLink. https://www.oncolink.org/cancer-treatment/therapies/targeted/part-one-introduction-to-small-molecule-tyrosine-kinase-inhibitors. Published 2010. Accessed February 16, 2017.

54. Druker BJ, Guilhot F, O'Brien SG, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med*. 2006;355(23):2408-2417. doi:10.1056/NEJMoa062867.

55. Cornelison AM, Kantarjian H, Cortes J, Jabbour E. Outcome of treatment of chronic myeloid leukemia with second-generation tyrosine kinase inhibitors after imatinib failure. *Clin Lymphoma Myeloma Leuk*. 2011;11 Suppl 1(0 1):S101-10. doi:10.1016/j.clml.2011.02.009.

56. Baccarani M, Saglio G, Goldman J, et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood*. 2006;108(6).

57. Kantarjian HM, Larson RA, Guilhot F, et al. Efficacy of imatinib dose escalation in patients with chronic myeloid leukemia in chronic phase. *Cancer*. 2009;115(3):551-560. doi:10.1002/cncr.24066.

58. Stein B, Smith BD. Treatment options for patients with chronic myeloid leukemia

who are resistant to or unable to tolerate imatinib. *Clin Ther*. 2010;32(5):804-820. doi:10.1016/j.clinthera.2010.05.003.

59. O'Hare T, Walters DK, Stoffregen EP, et al. In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res*. 2005;65(11).

60. Breccia M, Alimena G. Second-generation tyrosine kinase inhibitors (TKI) as salvage therapy for resistant or intolerant patients to prior TKIs. *Mediterr J Hematol Infect Dis*. 2014;6(1):e2014003. doi:10.4084/MJHID.2014.003.

61. Kantarjian H, Pasquini R, Lévy V, et al. Dasatinib or high-dose imatinib for chronic-phase chronic myeloid leukemia resistant to imatinib at a dose of 400 to 600 milligrams daily. *Cancer*. 2009;115(18):4136-4147. doi:10.1002/cncr.24504.

62. Apperley JF, Cortes JE, Kim D-W, et al. Dasatinib in the treatment of chronic myeloid leukemia in accelerated phase after imatinib failure: the START a trial. *J Clin Oncol*. 2009;27(21):3472-3479. doi:10.1200/JCO.2007.14.3339.

63. Cortes J, Kim D-W, Raffoux E, et al. Efficacy and safety of dasatinib in imatinib-resistant or -intolerant patients with chronic myeloid leukemia in blast phase. *Leukemia*. 2008;22(12):2176-2183. doi:10.1038/leu.2008.221.

64. Dasatinib - drug information - Chemocare. http://chemocare.com/chemotherapy/drug-info/dasatinib.aspx. Accessed February 21, 2017.

65. Deininger MW. Nilotinib. *Clin Cancer Res*. 2008;14(13).

66. Jabbour E, Hochhaus A, Cortes J, La Rosée P, Kantarjian HM. Choosing the best treatment strategy for chronic myeloid leukemia patients resistant to imatinib: weighing the efficacy and safety of individual drugs with BCR-ABL mutations and patient history. *Leukemia*. 2010;24(1):6-12. doi:10.1038/leu.2009.193.

67. Jabbour E, Kantarjian H, Cortes J. Use of second- and third-generation tyrosine kinase inhibitors in the treatment of chronic myeloid leukemia: an evolving treatment paradigm. *Clin Lymphoma Myeloma Leuk*. 2015;15(6):323-334. doi:10.1016/j.clml.2015.03.006.

68. Gurion R, Gafter-Gvili A, Vidal L, et al. Has the time for first-line treatment with second generation tyrosine kinase inhibitors in patients with chronic myelogenous leukemia already come? Systematic review and meta-analysis. *Haematologica*. 2013;98(1):95-102. doi:10.3324/haematol.2012.063172.

69. Tanneeru K, Guruprasad L. Ponatinib is a pan-BCR-ABL kinase inhibitor: MD simulations and SIE study. *PLoS One*. 2013;8(11):e78556.

doi:10.1371/journal.pone.0078556.

70. Cortes JE, Kantarjian H, Shah NP, et al. Ponatinib in refractory Philadelphia chromosome-positive leukemias. *N Engl J Med*. 2012;367(22):2075-2088. doi:10.1056/NEJMoa1205127.

71. Cortes JE, Kim D-W, Pinilla-Ibarz J, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. *N Engl J Med*. 2013;369(19):1783-1796. doi:10.1056/NEJMoa1306494.

72. Bhamidipati PK, Kantarjian H, Cortes J, Cornelison AM, Jabbour E. Management of imatinib-resistant patients with chronic myeloid leukemia. *Ther Adv Hematol*. 2013;4(2):103-117. doi:10.1177/2040620712468289.

73. Bradeen HA, Eide CA, O'Hare T, et al. Comparison of imatinib mesylate, dasatinib (BMS-354825), and nilotinib (AMN107) in an N-ethyl-N-nitrosourea (ENU)-based mutagenesis screen: high efficacy of drug combinations. *Blood*. 2006;108(7):2332-2338. doi:10.1182/blood-2006-02-004580.

74. Widmer N, Rumpold H, Untergasser G, Fayet A, Buclin T, Decosterd LA. Resistance reversal by RNAi silencing of MDR1 in CML cells associated with increase in imatinib intracellular levels. *Leukemia*. 2007;21(7):1561-1562. doi:10.1038/sj.leu.2404671.

75. White DL, Saunders VA, Dang P, et al. CML patients with low OCT-1 activity achieve better molecular responses on high dose imatinib than on standard dose. Those with high OCT-1 activity have excellent responses on either dose: A TOPS correlative study. *Blood*. 2015;112(11).

76. Gambacorti-Passerini C, Barni R, le Coutre P, et al. Role of alpha1 acid glycoprotein in the in vivo resistance of human BCR-ABL+ leukemic cells to the abl inhibitor STI571. *J Natl Cancer Inst*. 2000;92(20):1641-1650. doi:10.1093/jnci/92.20.1641.

77. Bixby D, Talpaz M. Mechanisms of resistance to tyrosine kinase inhibitors in chronic myeloid leukemia and recent therapeutic strategies to overcome resistance. *Hematol Am Soc Hematol Educ Progr*. 2009;2009(1):461-476. doi:10.1182/asheducation-2009.1.461.

78. Co-trimoxazole monograph for professionals - Drugs.com. American Society of Health-System Pharmacists, Inc. https://www.drugs.com/monograph/co-trimoxazole.html. Published 2013. Accessed March 1, 2017.

79. Schrøder H, Agger KE, Rosthøj S, Carlsen NT, Schmiegelow K. Antibacterial prophylaxis with trimethoprim-sulfamethoxazole during induction treatment for acute lymphoblastic leukemia. *Dan Med Bull*. 2001;48(4):275-277.

http://www.ncbi.nlm.nih.gov/pubmed/11767129. Accessed March 10, 2017.

80. Commissioner of the Safety Information. Septra and septra DS (trimethoprim and sulfamethoxazole) tablets. https://www.rxlist.com/septra-drug.html. Published 2013. Accessed March 10, 2017.

81. Sulfamethoxazole/trimethoprim (oral route) side effects - Mayo Clinic. http://www.mayoclinic.org/drugs-supplements/sulfamethoxazole-trimethoprim-oral-route/side-effects/drg-20071899. Accessed March 1, 2017.

82. Koch-Weser J, Rubin RH, Swartz MN. Trimethoprim-sulfamethoxazole. *N Engl J Med*. 1980;303(8):426-432. doi:10.1056/NEJM198008213030804.

83. Gentamicin sulfate monograph for professionals - Drugs.com. American Society of Health-System Pharacists, Inc. https://www.drugs.com/monograph/gentamicin-sulfate.html. Published 2013. Accessed March 1, 2017.

84. Gentamicin dosage guide with precautions. Drugs.com. https://www.drugs.com/dosage/gentamicin.html. Published 2017. Accessed March 10, 2017.

85. Rao SC, Srinivasjois R, Moon K. One dose per day compared to multiple doses per day of gentamicin for treatment of suspected or proven sepsis in neonates. In: Rao SC, ed. *Cochrane Database of Systematic Reviews*. Chichester, UK: John Wiley & Sons, Ltd; 2016. doi:10.1002/14651858.CD005091.pub4.

86. Ho KK, Bryson SM, Thiessen JJ, Greenberg ML, Einarson TR, Leson CL. The effects of age and chemotherapy on gentamicin pharmacokinetics and dosing in pediatric oncology patients. *Pharmacother J Hum Pharmacol Drug Ther*. 1995;15(6):754-764. doi:10.1002/J.1875-9114.1995.TB02893.X.

87. Mareville J, Gay J, Cliquennois E, et al. Therapeutic drug monitoring of aminoglycosides in acute myeloid leukaemia patients. *Scand J Infect Dis*. 2012;44(5):398-401. doi:10.3109/00365548.2011.645868.

88. Al-Omary FAM, Abou-zeid LA, Nagi MN, et al. Non-classical antifolates. Part 2: Synthesis, biological evaluation, and molecular modeling study of some new 2,6-substituted-quinazolin-4-ones. *Bioorg Med Chem*. 2010;18(8):2849-2863. doi:10.1016/j.bmc.2010.03.019.

89. Vydrin AF, Shikhaleev I V., Makhortov VL, Kolchanova N V. Component composition of gentamicin sulfate preparations. *Pharm Chem J*. 2003;37(8):448-450. doi:10.1023/A:1027372416983.

90. Isoherranen N, Lavy E, Soback S. Pharmacokinetics of gentamicin C(1), C(1a), and C(2) in beagles after a single intravenous dose. *Antimicrob Agents Chemother*.

2000;44(6):1443-1447. http://www.ncbi.nlm.nih.gov/pubmed/10817690. Accessed March 1, 2017.

91. Ceftazidime monograph for professionals - Drugs.com. American Society of Health-System Pharmacists, Inc. https://www.drugs.com/monograph/ceftazidime.html. Published 2013. Accessed March 1, 2017.

92. Pea F, Viale P, Damiani D, et al. Ceftazidime in acute myeloid leukemia patients with febrile neutropenia: helpfulness of continuous intravenous infusion in maximizing pharmacodynamic exposure. *Antimicrob Agents Chemother*. 2005;49(8):3550-3553. doi:10.1128/AAC.49.8.3550-3553.2005.

93. Ceftazidime. National Center for Biotechnology Information. PubChem Compound Database. CID=5481173, https://pubchem.ncbi.nlm.nih.gov/compound/5481173. Accessed March 1, 2017.

94. Hayes M V, Orr DC. Mode of action of ceftazidime: affinity for the penicillin-binding proteins of Escherichia coli K12, Pseudomonas aeruginosa and Staphylococcus aureus. *J Antimicrob Chemother*. 1983;12(2):119-126. http://www.ncbi.nlm.nih.gov/pubmed/6413485. Accessed March 1, 2017.

95. Viktil KK, Blix HS, Moger TA, Reikvam A. Polypharmacy as commonly defined is an indicator of limited value in the assessment of drug-related problems. *Br J Clin Pharmacol*. 2007;63(2):187-195. doi:10.1111/j.1365-2125.2006.02744.x.

96. Meibohm B, Derendorf H. Basic concepts of pharmacokinetic/pharmacodynamic (PK/PD) modelling. *Int J Clin Pharmacol Ther*. 1997;35(10):401-413. http://www.ncbi.nlm.nih.gov/pubmed/9352388. Accessed February 28, 2017.

97. Palleria C, Di Paolo A, Giofrè C, et al. Pharmacokinetic drug-drug interaction and their implication in clinical management. *J Res Med Sci*. 2013;18(7):601-610. http://www.ncbi.nlm.nih.gov/pubmed/24516494. Accessed July 11, 2017.

98. Riechelmann RP, Tannock IF, Wang L, Saad ED, Taback NA, Krzyzanowska MK. Potential drug interactions and duplicate prescriptions among cancer patients. *J Natl Cancer Inst*. 2007;99(8):592-600. doi:10.1093/jnci/djk130.

99. Haouala A, Widmer N, Duchosal MA, Montemurro M, Buclin T, Decosterd LA. Drug interactions with the tyrosine kinase inhibitors imatinib, dasatinib, and nilotinib. *Blood*. 2011;117(8).

100. Cholongitas E, Pipili C, Katsogridakis K, Relos K, Dasenaki M. Dermatitis after suspected imatinib–levothyroxine interaction in a patient with gastrointestinal stromal tumor. *Cancer Chemother Pharmacol*. 2008;61(6):1083-1084. doi:10.1007/s00280-007-0562-0.

101. Gambillara E, Laffitte E, Widmer N, et al. Severe pustular eruption associated with imatinib and voriconazole in a patient with chronic myeloid leukemia. *Dermatology*. 2005;211(4):363-365. doi:10.1159/000088510.

102. Zhou S-F, Xue CC, Yu X-Q, Li C, Wang G. Clinically important drug interactions potentially involving mechanism-based inhibition of cytochrome P450 3A4 and the role of therapeutic drug monitoring. *Ther Drug Monit*. 2007;29(6):687-710. doi:10.1097/FTD.0b013e31815c16f5.

103. Bolton AE, Peng B, Hubert M, et al. Effect of rifampicin on the pharmacokinetics of imatinib mesylate (Gleevec, STI571) in healthy subjects. *Cancer Chemother Pharmacol*. 2004;53(2):102-106. doi:10.1007/s00280-003-0722-9.

104. Sparano BA, Egorin MJ, Parise RA, et al. Effect of antacid on imatinib absorption. *Cancer Chemother Pharmacol*. 2009;63(3):525-528. doi:10.1007/s00280-008-0778-7.

105. FRYE R, FITZGERALD S, LAGATTUTA T, HRUSKA M, EGORIN M. Effect of St John's wort on imatinib mesylate pharmacokinetics. *Clin Pharmacol Ther*. 2004;76(4):323-329. doi:10.1016/j.clpt.2004.06.007.

106. Busti AJ. The mechanism for trimethoprim/sulfamethoxazole's (Bactrim; Bactrim DS; Septra) inhibition of warfarin (Coumadin; Jantoven). http://www.ebmconsult.com/articles/tmp-smx-bactrim-septra-warfarin-coumadin-interaction-mechanims. Published 2015. Accessed March 10, 2017.

107. O'Brien SG, Meinhardt P, Bond E, et al. Effects of imatinib mesylate (STI571, Glivec) on the pharmacokinetics of simvastatin, a cytochrome p450 3A4 substrate, in patients with chronic myeloid leukaemia. *Br J Cancer*. 2003;89(10):1855-1859. doi:10.1038/sj.bjc.6601152.

108. Khalili H, Bairami S, Kargar M. Antibiotics induced acute kidney injury: incidence, risk factors, onset time and outcome. *Acta Med Iran*. 2013;51(12):871-878. http://www.ncbi.nlm.nih.gov/pubmed/24442542. Accessed March 10, 2017.

109. Imatinib - FDA prescribing information, side effects and uses. Teva Pharmaceuticals USA, Inc. https://www.drugs.com/pro/imatinib.html#ID_86707d79-bb51-4508-ae18-5c94371bed8f. Published 2016. Accessed March 10, 2017.

110. Peng B, Lloyd P, Schran H. Clinical pharmacokinetics of imatinib. *Clin Pharmacokinet*. 2005;44(9):879-894. doi:10.2165/00003088-200544090-00001.

111. Broadway N. How to develop assays: 5 considerations and 8 fundamentals. *Mater Methods*. 2012;2. doi:10.13070/mm.en.2.121.

112. Little TA. Assay Development and method validation essentials. http://www.biopharminternational.com/assay-development-and-method-validation-essentials. Accessed July 13, 2017.

113. Padmalayam I. Assay development in drug discovery. https://www.uab.edu/medicine/adda/images/PadmalayamAssayDevelopment.pdf. Accessed July 13, 2017.

114. Riss TL, Moravec RA, Niles AL, et al. Cell viability assays. Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004. http://www.ncbi.nlm.nih.gov/pubmed/23805433. Accessed July 13, 2017.

115. K-562 ATCC ® CCL-243™ Homo sapiens bone marrow chronic myelogenous leukemia. https://www.atcc.org/products/all/CCL-243.aspx. Published 2016. Accessed July 24, 2017.

116. Strober W, Strober, Warren. Trypan blue exclusion test of cell viability. In: *Current Protocols in Immunology*. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2015:A3.B.1-A3.B.3. doi:10.1002/0471142735.ima03bs111.

117. CellTiter-Glo® luminescent cell viability assay protocol. https://www.promega.com/resources/protocols/technical-bulletins/0/celltiter-glo-luminescent-cell-viability-assay-protocol/. Accessed June 21, 2017.

118. Aubert G, Carricajo A, Coudrot M, Guyomarc&#39 S, Auboyer C, Zeni F. Prospective determination of serum ceftazidime concentrations in intensive care units. *Ther Drug Monit*. 2010;32(4):517-519. doi:10.1097/FTD.0B013E3181E60CA6.

119. Hammett-Stabler CA, Johns T. Laboratory guidelines for monitoring of antimicrobial drugs. *Clin Chem*. 1998;44(5). http://clinchem.aaccjnls.org/content/44/5/1129.long. Accessed June 1, 2017.

120. Dudley MN, Levitz RE, Quintiliani R, Hickingbotham JM, Nightingale2 CH. Pharmacokinetics of trimethoprim and sulfamethoxazole in serum and cerebrospinal fluid of adult patients with normal meninges. *Antimicrob Agents Chemother*. 1984:811-814. http://aac.asm.org/content/26/6/811.full.pdf. Accessed June 1, 2017.

121. Zhang G, Li M, Han S, et al. Induction of human chronic myeloid leukemia K562 cell apoptosis by virosecurinine and its molecular mechanism. *Mol Med Rep*. 2014;10(5):2365-2371. doi:10.3892/mmr.2014.2531.

122. Balakrishna A, Kumar MH. Evaluation of synergetic anticancer activity of berberine and curcumin on different models of A549, Hep-G2, MCF-7, Jurkat, and K562 Cell Lines. *Biomed Res Int*. 2015;2015:354614. doi:10.1155/2015/354614.

123. Galvao J, Davis B, Tilley M, Normando E, Duchen MR, Cordeiro MF. Unexpected low-dose toxicity of the universal solvent DMSO. *FASEB J*. 2014;28(3):1317-1330. doi:10.1096/fj.13-235440.

124. Hamzeloo-Moghadam M, Taiebi N, Mosaddegh M, Tehrani BE, Esmaeili S. The effect of some cosolvents and surfactants on viability of cancerous cell lines. *Res J Pharmacogn*. 1(2014):41-45. http://www.rjpharmacognosy.ir/pdf_5777_9dc5c5256a5d2af01f9cf25200e897e9.html. Accessed June 27, 2017.

125. Kang Y, Hodges A, Ong E, Roberts W, Piermarocchi C, Paternostro G. Identification of drug combinations containing imatinib for treatment of BCR-ABL+ leukemias. *PLoS One*. 2014;9(7):e102221. doi:10.1371/journal.pone.0102221.

126. Zhang J-H, He Y-L, Zhu R, Du W, Xiao J-H. Deregulated expression of Cdc6 as BCR/ABL-dependent survival factor in chronic myeloid leukemia cells. *Tumor Biol*. 2017;39(6):101042831771339. doi:10.1177/1010428317713394.

127. Yamakawa Y, Hamada A, Uchida T, et al. Distinct interaction of nilotinib and imatinib with P-Glycoprotein in intracellular accumulation and cytotoxicity in CML cell line K562 cells. *Biol Pharm Bull*. 2014;37(8):1330-1335. doi:10.1248/bpb.b14-00254.

128. Wu R, Huang Y, Ma X. Effect of phenylhexyl isothiocyanate on drug-resistance to imatinib in K562/G01 cell line. *Zhonghua Xue Ye Xue Za Zhi*. 2013;34(2):149-152. http://www.ncbi.nlm.nih.gov/pubmed/23611223. Accessed June 28, 2017.

129. Lee Y-L, Chen C-W, Liu F-H, Huang Y-W, Huang H-M. Aclacinomycin A sensitizes K562 chronic myeloid leukemia cells to imatinib through p38MAPK-mediated erythroid differentiation. *PLoS One*. 2013;8(4):e61939. doi:10.1371/journal.pone.0061939.

130. van Tonder A, Joubert AM, Cromarty AD. Limitations of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. *BMC Res Notes*. 2015;8:47. doi:10.1186/s13104-015-1000-8.

131. Hanson KL, Cartwright CP. Evaluation of an automated liquid-handling system (Tecan Genesis RSP 100) in the Abbott LCx assay for Chlamydia trachomatis. *J Clin Microbiol*. 2001;39(5):1975-1977. doi:10.1128/JCM.39.5.1975-1977.2001.

132. Stangegaard M, Froslev TG, Frank-Hansen R, Hansen AJ, Morling N. Automated extraction of DNA from blood and PCR setup using a Tecan Freedom EVO liquid handler for forensic genetic STR yyping of reference samples. *J Lab Autom*. 2011;16(2):134-140. doi:10.1016/j.jala.2010.11.003.

133. Jones RE, Zheng W, McKew JC, Chen CZ. An alternative direct compound dispensing method using the HP D300 digital dispenser. *J Lab Autom*. 2013;18(5):367-374. doi:10.1177/2211068213491094.

134. Kang Y, Hodges A, Ong E, Roberts W, Piermarocchi C, Paternostro G. Identification of drug combinations containing imatinib for treatment of BCR-ABL+ leukemias. *PLoS One*. 2014;9(7):e102221. doi:10.1371/journal.pone.0102221.

135. Yusuf B, Oztekin C, Yonca BE. Inhibition of proteasome by bortezomib increase chemosensitivity of bcr/abl positive human k562 chronic myleoid leukemia cells to imatinib. *Health (Irvine Calif)*. 2009;1(4):320-324. doi:10.4236/health.2009.14052.

136. Salih J, Hilpert J, Placke T, et al. The BCR/ABL-inhibitors imatinib, nilotinib and dasatinib differentially affect NK cell reactivity. doi:10.1002/ijc.25233.

137. Cassuto O, Dufies M, Jacquel A, et al. All tyrosine kinase inhibitor-resistant chronic myelogenous cells are highly sensitive to ponatinib. *Oncotarget*. 2012;3(12):1557-1565. doi:10.18632/oncotarget.692.

138. Husaini R, Ahmad M, Zakaria Z. Effectiveness of imatinib mesylate over etoposide in the treatment of sensitive and resistant chronic myeloid leukaemia cells in vitro. *Exp Ther Med*. 2017;13(6):3209-3216. doi:10.3892/etm.2017.4443.

139. Hegedu ″s C, Özvegy-Laczka C, Apáti Á, et al. Interaction of nilotinib, dasatinib and bosutinib with ABCB1 and ABCG2: implications for altered anti-cancer effects and pharmacological propertiesb. *Br J Pharmacol*. 2009;158:1153-1164. doi:10.1111/j.1476-5381.2009.00383.x.

140. Luo FR, Yang Z, Camuso A, et al. Dasatinib (BMS-354825) pharmacokinetics and pharmacodynamic biomarkers in animal models predict optimal clinical exposure. doi:10.1158/1078-0432.CCR-06-1112.

141. Radujkovic A, Schad M, Topaly J, et al. Synergistic activity of imatinib and 17-AAG in imatinib-resistant CML cells overexpressing BCR-ABL – Inhibition of P-glycoprotein function by 17-AAG. *Leukemia*. 2005;19(7):1198-1206. doi:10.1038/sj.leu.2403764.

142. Aras Y, Erguven M, Aktas E, Yazihan N, Bilir A. Antagonist activity of the antipsychotic drug lithium chloride and the antileukemic drug imatinib mesylate during glioblastoma treatment *in vitro*. *Neurol Res*. 2016;38(9):766-774. doi:10.1080/01616412.2016.1203096.

143. Drexler HG, Macleod RAF, Uphoff CC. Leukemia cell lines: in vitro models for the study of Philadelphia chromosome-positive leukemia. *Leuk Res*. 1999;23:207-

215. http://ac.els-cdn.com.ezproxy.lib.utah.edu/S0145212698001714/1-s2.0-S0145212698001714-main.pdf?_tid=5dca75ac-4708-11e7-b604-00000aab0f6b&acdnat=1496348871_3fd18ba12a62234c60770cda492b4610. Accessed June 1, 2017.

144. Lozzio C, Lozzio B. Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. *Blood*. 1975;45(3). http://www.bloodjournal.org.ezproxy.lib.utah.edu/content/45/3/321.long?sso-checked=true. Accessed June 1, 2017.

145. Naumovski L, Morgan R, Hecht F, Link MP, Glader BE, Smith SD. Philadelphia chromosome-positive acute lymphoblastic leukemia cell lines without classical breakpoint cluster region rearrangement. *Cancer Res*. 1988;48(10). http://cancerres.aacrjournals.org.ezproxy.lib.utah.edu/content/48/10/2876.long. Accessed June 1, 2017.