Spring 2019

# DUAL THERAPY TREATMENT OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA WITH BLINOTUMOMAB AND A STANDARD CHEMOTHERAPY REGIMEN

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By

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Submitted to the School of Honors Committee

in partial fulfillment

of the requirements for University Honors Scholars

Southeastern University

2019

# Dedication

I would like to dedicate this to all of the families with children fighting the seemingly never-ending battle against cancer. I pray and long for the day where the word cancer no longer holds the level of fear behind it that it does now. May the peace of Christ rule your hearts and minds, and may you be blessed with immeasurable strength.

Acknowledgements

I am incredibly thankful for the individuals the Lord has placed in my life throughout my time at Southeastern University. I would like to thank my family for encouraging me to attend this university, and for their constant support. Mom and Dad, thank you for never forgetting to tell me how proud of me you are, it has meant more than you know over the years! I would also like to thank all of the faculty for their dedication to students. Thank you, Dr. Schraw, my thesis advisor, for taking the time to explain to me some of the deepest scientific concepts while I am working in your office, followed by a quick sermon as to how that points to the Lord and His goodness and faithfulness. You have reminded me of the immeasurable mercies and grace God has given us and privilege it is to be a servant of Christ in the field of medicine! I would also like to thank Dr. Franklin for her constant support and encouragement. Thank you for reminding me of the beauty of being good enough, of appreciating the waiting place, and for giving me my first five hundred miles. It means mean more than you know! Thank you, Dr. Miller, for the love you have for all the Honors students, it does not go unnoticed and we are so fortunate to have a leader like you. I would also like to thank all of my friends, both science and non-science majors, who have encouraged me and played a role in shaping my college years. I would not have enjoyed and appreciated these four years without you all, and I look forward to the day when I am able to serve overseas in medical camps with you, lead in a church with you, and take the gospel to the nations with you. Finally, I would like to thank the Lord for giving me a heart and desire to practice medicine and serve His people in this field. I am humbled to have this opportunity, and I pray that you would be glorified through every year of school and residency, through every patient seen, and through every story I have the privilege of hearing and being a part of.

**Abstract:** Leukemia is the number one cancer affecting children in the nation, with acute lymphoblastic leukemia being the most prevalent classification.[1] While new and innovative treatment protocols have greatly increased the success rate of primary cancer patients, those who face relapse receive a much more dismal prognosis. Recent studies have shown that patients who relapse quite frequently have developed drug-resistant clones of the original cancer cells, leading to a need for various secondary treatment options. The drug-resistance is due to clonal mutations that take place within the cancer cell, most often because of an outside pressure or stress within the environment of the cell. In fact, studies show that in many cases the chemotherapy and radiation treatment administered to the leukemia patients provides the added pressures necessary to promote these clonal mutations, leading to treatment-resistant cells and the onset of relapsed leukemia. Various immunotherapies are becoming the front-line secondary treatment option due to their high success rates and innovative techniques. Monoclonal antibodies such as Blinatumomab or Inatuzumab are currently the primary targets of research for acute lymphoblastic leukemia secondary therapy. However, dual therapy treatments of cancers have shown increased rates of event-free survival, overall survival, and progression-free survival, as well as decreased rates of drug resistant cancer cells. Therefore, the goal of this review is to promote the application of dual treatment therapy on relapsed ALL cells for improved outcome, as well as on primary ALL cells for decreased drug resistance.

**Key Words:** acute lymphoblastic leukemia, pediatric, monoclonal antibody, Blinatumomab, chemotherapy, dual therapy, relapse, clonal mutation, drug-resistance

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# DUAL THERAPY TREATMENT OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA WITH BLINOTUMOMAB AND A STANDARD CHEMOTHERAPY REGIMEN

## Introduction

Imagine that you are working to solve an incredibly complex problem, one that takes you years of hard work, and then finally after multiple heart-wrenching failures you determine a possible route that shows promising leads to an answer. Then, when you at last feel like a solution is within reach, a new variable is thrown into the problem that introduces a completely unforeseen challenge in reaching your end goal. This is oftentimes exactly what the frustrating battle with cancer can feel like.

There is an abundant selection of promising therapies and treatments for many common cancers and malignancies, but unfortunately, many of them still result in the cancer returning with a new set of mutated genes that are prepared to fight even harder than before. Rare cancers as well face many difficult obstacles to overcome when formulating a treatment plan. It can be a disheartening battle when treatment initially rids the body of malignant cells, but then new cancerous cells develop that are specifically made to resist the treatment that was once so promising.[1-3] This is why one of the greatest gifts that can be given to patients with these devastating afflictions is time. However, with many advances in technology today, there is potential to outsmart the cancer cells before they have the opportunity to mutate and deceive the body. With the implementation of dual-therapy treatments in many varieties of cancer, survival rates have significantly increased.[3] There have also been noticeable decreases in rates of relapse[3], which is one of the key indicators measured when analyzing overall survival for cancer patients. If there was an opportunity for a treatment that not only aided a patient's immune system in fighting off cancer cells, but also provided a potential for a lower risk of relapse, all

measures should be taken, and all costs be funded in order to achieve this goal. The capability of a treatment to increase the patient's quality of life and life expectancy should be a primary goal in cancer research. Presented within this thesis is a proposal to combine a standard chemotherapy regimen, which has been shown to limit cancer cell growth and expansion, with an innovative monoclonal antibody named Blinatumomab to directly target the patient's immune response cells to initiate the desired apoptotic effect on cancerous acute lymphoblastic leukemia cells. Blinatumomab is considered an immunotherapeutic agent because of its ability to empower the patient's own immune system to defeat the cancer cells. It has shown great promise in previous studies with acute lymphoblastic leukemia patients, and in combination with chemotherapy could be a lifechanging treatment for these children.[1,2] In order to accurately determine the potential for a dual therapy treatment to effectively treat relapsed leukemia patients, as well as to decrease rates of relapse in primary cancer diagnosed patients, several tests should be completed. The goals of each test are to examine the efficacy of a dual therapy treatment in comparison to a monotherapy treatment for both primary and secondary leukemia, as well as to research a potential treatment for a population of cancer patients that do not contain the receptor that Blinatumomab targets. If the tests give promising results, then the next goal would be to move on to clinical trials to determine if the treatments are effective and safe for the human population.

**Background and Significance**

Leukemia, a well-known disease affecting the blood cells, is the number one cancer in the pediatric population, and the second cause of death in children age's 0-14.[3] There are four classifications of leukemia including Chronic Myeloid, Chronic Lymphoblastic, Acute Myeloid, and Acute Lymphoblastic. The most prevalent form in children is Acute Lymphoblastic Leukemia (ALL), which is diagnosed in approximately 3,800 patients each year, comprising nearly eighty percent of all ALL cases diagnosed annually.[4,5] Since the discovery of leukemia in the 19th century, the methods of treatment for this significant number of patients have improved considerably, leading to a substantial increase in patient overall survival rates.[3]

In 1847 the disease was named by Dr. Rudolf Virchow, a German politician who spent a large part of his time researching cell biology, pathology and anthropology.[6] However, the first published case of the disease was in 1899 when Major Samuel T. Armstrong, a military surgeon, died of the disease in Manila.[6] By 1913 there had been several more case reports of deaths caused by leukemia, as well as the discovery of the different classifications of the disease. The first mention of treatment was in December of 1913, when physicians attempted to treat a student from Cornell University by performing a blood transfusion with his twin brother, which was unsuccessful.[6] Two years later, doctors discovered that radium was successful in treating patients, but the poison had severe negative effects on the body systems. However, radiation therapy is still a widely used treatment for the cancer today. By the 1930's the disease had been frequently mentioned, but was still left without a cure, other than futile attempts at blood transfusions. In 1946 the treatment of leukemia with chemicals was first mentioned in *The New York Times* after an anonymous reporter shared that chemical medications rejected for the treatment of malaria, because they destroyed white blood cells, may be beneficial for anti-

leukemia therapies.[6] Today, there is still no defined cure for leukemia, but treatments such as chemotherapy, stem cell transplants, and new immunotherapies have significantly increased the survival rate of diagnosed patients.[3]

The cause of leukemia varies from patient to patient, but like any cancer there are various factors that can increase the likelihood of developing the disease. Recent studies have shown that multiple genetic syndromes can predispose a patient to developing ALL, including Down syndrome, Fanconi anemia, Bloom syndrome, ataxia telangiectasia, and Nijmegen breakdown syndrome.[5] Other predisposing factors include exposure to pesticides, ionizing radiation, or certain viruses such as Epstein-Barr Virus or Human Immunodeficiency Virus.[5] However, in the majority of diagnoses the disease develops de novo in previously healthy individuals. ALL can be further divided into two main classifications, namely, B cell ALL and T cell ALL. Seventy-five to eighty-five percent of all pediatric patients with ALL have cancer cells that developed from the B cell origin, leading to the arrest of an immature B cell progenitor.[7] B-ALL typically develops in utero as a result of a chromosomal translocation, of which there are many variations.[7] This abnormality causes a differentiation arrest in developing B lymphoid cells, leading to their suspension in a differentiated state before having reached maturity, or in some cases they are reverted to a pre-differentiation state and then arrested, causing them to rapidly proliferate as leukemia blasts.[8,9] The leukemia blasts will then advance from the bone marrow into the bloodstream to penetrate the spleen, liver, central nervous system, thymus and lymph nodes.[2] This takeover of the hematopoietic system also has detrimental effects on red blood cells and platelets by significantly decreasing their counts within the peripheral blood.[8] The negative effects on the hematopoietic system then have further consequences on other body systems such as the immunological and physiological systems, many times leading to death.[8] There are many

genetic abnormalities leading to the occurrence of acute lymphoblastic leukemia, including the formation of the Philadelphia chromosome (see Figure 1). This is typically cause by the chromosomal fusion of the BCR and ABL genes from chromosomes 9 and 22, producing a significantly shorter chromosome as well as significant health defects.[10] This fusion leads to the activation of downstream kinases within multiple signaling pathways which causes cells to stray from their normal signal response, thereby causing proliferation and oncogenesis of leukemia, many times being initiated in the myeloid compartment.[11] The myeloid compartment is a part of the immune system and contains a large mixed group of myeloid-stemmed cells including neutrophils, eosinophils, dendritic cells, and mast cells.[12] The problem with the interaction of the myeloid compartment with the Philadelphia chromosome is that all of cells contained within this section can be affected by the Philadelphia mutation, thereby causing a greater distribution throughout the body.[13] The myeloid cells within the myeloid compartment, when influenced by cancerous cells, play a large role in the metastatic process of cancer development, from delamination from the primary tumor mass to the invasion of neighboring tissue to eventual colonization of the site of metastasis.[12] However, in many cases the genetic alterations are not enough to cause the onset of the disease, and therefore a second factor is required. Potential secondary contributors to leukemia are currently being studied and include environmental, ethnic, immunologic, infectious, and socioeconomic components.[7]

Treatment of acute lymphoblastic leukemia has greatly improved over the years with the advent of multidrug, risk-adapted chemotherapy regimens, as well as recognition of treatment response characteristics that can identify patients at risk for treatment failure.[14] This has led to cure rates of approximately 90%, an astounding statistic, however for the 10-20% of pediatric patients who relapse the outlook is dismal and has shown little to no improvement in the last

twenty years.[15] Current treatment for relapsed ALL is surprisingly similar to primarily diagnosed patients with the exception that treatment is typically dose intensified or given on alternative schedules.[14] Once a patient reaches second complete remission (CR2) they will usually continue intensive chemotherapy treatment. Furthermore, a hematopoietic stem cell transplant is also a potential treatment for the secondary cancer. However, these treatment strategies have not led to significantly improved overall survival (OS) rates, especially with relapsed cancers with poor prognostic factors such as early bone marrow relapse, thereby leading to the need for novel treatment therapies.[14]

Currently the best primary treatment for patients originally diagnosed with ALL is a regimen of chemotherapy with the goal of reaching induction, followed by consolidation and then maintenance therapy.[14] The best indicator of long-term survival is the quantity of time a patient survives disease-free.[16] Patients that relapse within one year of being declared disease-free maintain the most dismal prognosis of long-term survival.[17] Therefore, it is vital to implement treatment options that decreased rates of relapse within patients to give them a great chance at long-term survival.

*Primary Treatment Options*

With the increasing percentage of annual leukemia diagnoses in the United States, a standard treatment protocol for the cancer is required. Typically, when a patient is initially diagnosed with acute lymphoblastic leukemia, they will undergo a three-phase cancer treatment process as mentioned above. These three steps include induction (or remission induction), consolidation (or intensification), and then maintenance therapy.[18] The goal of the induction phase is remission, which is defined as when leukemia cells are no longer found in bone marrow

tissue samples, normal marrow cells return, and the blood counts become normalized.[18] This does not mean, however, that the patient is "cured", because there may still be remnant cancer cells undetected within the patient's body. Typical chemotherapy drugs administered during the induction phase include Vincristine, Dexamethasone or prednisone, or Doxorubicin.[18] Doxorubicin is an anthracycline antibiotic administered intravenously through either a central or peripheral venous line.[19] Furthermore, Doxorubicin is also a vesicant, meaning that it can cause severe tissue damage if it escapes from the vein.[19] Doxorubicin is an antitumor antibiotic, meaning that it is derived from a natural product and acts during multiple phases of the cell-cycle, thereby making it a cell-cycle specific drug.[19] Chemotherapy drugs such as Doxorubicin are not cancer specific and can target any rapidly dividing cell, leading to common side effects such as hair loss (alopecia), mouth sores, nausea, and diarrhea.[19] Dexamethasone is a second chemotherapeutic drug commonly used for treatment of ALL. It is a glucocorticosteroid that is distributed in pill form, many times alongside other chemotherapy drugs.[20] It is an anti-inflammatory frequently used in the short-term treatment of nausea caused by other chemotherapy drugs, but it has also been found to cause apoptosis, and can therefore aid in the fight against the cancerous cells.[20] Finally, Vincristine is a third drug commonly used in treatment for ALL among other cancers.[21] It is also a vesicant that is given intravenously, and risks the same side effects as Doxorubicin.[21] Furthermore, it belongs to a class of chemotherapy drugs called plant alkaloids, and is specifically a member of the vinca alkaloids group. Vinca alkaloids are microtubule agents that inhibit the microtubule structures within a cell, preventing proper division and replication, and eventually leading to cell death.[21] However, chemotherapy agents can be extremely toxic to healthy tissue as well as cancer cells, and so it would be

incredibly beneficial to propose treatment options that minimize use of vesicant chemotherapies, or eliminate them completely.

*Incidence of Relapse*

While current treatment therapies for acute lymphoblastic leukemia have led to the astonishing survival of 90% of patients, the prognosis for the 10-20% that face relapse is oftentimes discouraging.[2] Chromosomal abnormalities, genetic mutations, and decreased responsiveness to chemotherapy are but a few of the differences seen between primarily diagnosed leukemia and the relapsed cancer.[22] A study done by Mullighan et al.[22] researched the genetic basis of relapse by performing DNA copy number analyses on both diagnosis and relapse samples from acute lymphoblastic leukemia patients. The results showed a majority of patients had different copy number abnormalities (CNAs) between relapse and diagnosis, specifically relapse samples lacked some of the CNAs identified at diagnosis, leading to the conclusion that genetic anomalies that lead to ALL relapse are brought about during therapeutic treatment and that the signaling pathways affected by these modifications may be promising targets for intervention.[22] Furthermore, a study done by Greaves et al. also showed how therapeutic treatment may inadvertently cause resistant variations in the cancer cells to evolve while the original clonal cells are being exterminated.[23] Studying matched pair samples from the same patient has allowed for observation of the biological pathways responsible for the drug resistant phenotype seen at relapse.[24,25] Copy number analyses (CNA) were performed by various researchers to detect copy number variations (CNVs), which are genomic alterations leading to an abnormal number of copies of one or more genes, due to either deletions, duplications, translocations or inversions.[26] Previous studies have shown that there are distinct genetic alterations between diagnosed leukemia cells and relapsed blasts, and comparison of the two

samples by CNA has also provided the opportunity to study the clonal evolution over time and map its origin.[24] In fact, studies show that the vast majority (approximately 94%) of relapses are derived from a clone present at diagnosis, while a small minority (close to 6%) occur from a genetically new leukemia.[14] Nearly 34% of these clones derived from the original cancer were present at diagnosis, while close to 52% evolved from the ancestral clone, as seen in Figure 2.[14] Furthermore, the CNA revealed that focal deletions were more common than additions, specifically deletions of *IKZF1, EBF1, BTG1, TBL1XR1,* and *MSH6*.[14] *IKZF1* is a gene responsible for encoding the lymphoid TF IKAROS, and deletion of this gene has been defined as a strong indicator of relapse.[27] Other deletions such as *NR3C1, BTG1,* and *TBL1XR1* have been identified in B-ALL as factors leading to glucocorticosteroid resistance.[28] Along with genetic deletions present in mutations at relapse, there are also multiple signaling pathways that can develop genomic lesions, leading to resistance to nearly all drug therapies. Studies done that performed integrated genomic profiling revealed that the activation of the WNT and MAPK pathways are frequently observed at relapse, and therefore could be a potential target for treatment.[29]

Clonal evolution following therapeutic treatment is becoming an increasingly recognized topic to begin to study the effects of chemotherapy and radiation treatment on cancer cells, not just as eradicators of the clonal cells rapidly proliferating, but also as amplifiers of clonal mutations causing resistance to further therapeutic treatments.[30,31]

*Secondary Treatment Options*

Immunotherapy is arguably the most promising leukemia treatment currently being studied.[14] The goal of immunotherapy is to aid the patients' immune system in fighting off

leukemic clones rather than merely administering exogenous chemicals to destroy the rapidly dividing cells. Two of the most increasingly popular immunotherapy methods being studied are CAR-T cells and monoclonal antibodies. Chimeric antigen receptor T cell (CAR-T cell) therapy works by engineering T lymphocytes from the patients' blood with synthetic receptors made to recognize and eliminate specific cancer cells.[32] T cells are initially collected from the peripheral blood by leukapheresis, followed by apheresis. The separated T cells are then transfected with a viral or non-viral vector containing the CAR genome, and then expanded and purified.[32] Finally, the cells are tested for quality and sterility, which is a process that takes approximately two to four weeks (see Figure 3) Before the patient is injected with the resulting cells, they must undergo a lymphodepletion treatment.[32] CAR-T cells have been found to be most beneficial in the treatment of hematological malignancies such as leukemias, lymphomas, and myelomas . They are also most effective as anti-CD19 receptors, due to the fact that CD19 is a cell marker characterized with high expression in B-ALL cells.[32] A recent study done by Jae et al. analyzed the use of CAR-T cell therapy on fifty-three patients with relapsed B-ALL.[33] Patients received an infusion of T-cells expressing 19-28z CARs, meaning that the chimeric receptor was composed of an anti-CD19 antibody binding site as well as intracellular domains from the T-cell coactivating receptors CD28 and CD3-zeta chain.[33] The results showed that a total of 83% of patients reached complete remission (CR), with an overall survival of 12.9 months.[33] The two most toxic side effects observed with CAR-T cell treatment were cytokine release syndrome (CRS) and neurotoxicity.[33]

Blinatumomab is a monoclonal antibody (mAb) that has been reported as the most promising agent of innovative ALL treatment currently being studied. It is a bispecific T cell engager (BiTE) that uses one arm of the antibody (Ab) to bind to CD19 on B cell leukemia cells.

CD19 is one of the most commonly expressed cell surface markers on cancerous lymphoblasts. The second arm of the Ab binds to healthy $CD3^+$ T cells to facilitate the upregulation of T cell activation and increased cytotoxicity and apoptosis of the cancer cell.[34] The mechanism through which Blinatumomab works can be seen in Figure 4.[35] With the binding of each arm of the bispecific antibody, it allows for the juxtaposition of $CD3^+$ T-cells to malignant B-cells, which leads to the production of granzymes and perforins, thereby initiating apoptosis.[36]

One of the main consequences of treatment with both CAR-T cells and Blinatumomab is cytokine release syndrome (CRS). Specifically, the increase in IL-6, IL-10, and IFN-γ.[37] Clinical manifestations of CRS include high fever, hypotension, hypoxia, and respiratory distress.[38] Furthermore, organ dysfunctions can also occur, including liver transaminitis and renal insufficiency, as well as other life-threatening complications.[38] However, Blinatumomab is known to have lower rates of CRS in comparison to CAR-T cell therapy.[38] Due to the high increase in expression of IL-6 with CRS, inhibitors specifically for IL-6 have been studied and shown to dramatically improve effects of CRS.[37,38]

Blinatumomab has recently been approved by the FDA for the treatment of acute lymphoblastic leukemia, and has shown increased rates of event-free survival, disease-free survival, and progression-free survival in comparison to standard chemotherapy treatment alone.[39] However, dual therapy treatments have shown even greater results, especially monoclonal antibody and chemotherapy combination therapy. Therefore, the dual therapy treatment of acute lymphoblastic leukemia with Blinatumomab and chemotherapy is a potential route to increase survival rates in pediatric patients.

*Dual Therapy Treatments*

Dual therapy treatment of various cancers is becoming a widely recognized and encouraged tactic for the battle against cancer. While monotherapy regimens have shown promising results and are sufficient for the treatment of some patients, there are numerous limitations to response rates and duration of therapy.[40] The goal of combination therapy is to make the target cells more immunogenic, thereby increasing the effects of the immunotherapy when given with other treatment regimens. There are several methods by which immunotherapy makes a tumor cell more immunogenic. The first is by increasing the antigen and MHC Class I expression on the cancer cell to increase likelihood of T-cells binding to the cell to initiate cell-mediated apoptosis.[40] Another method is by altering the environment around the tumor cell by either increasing vascular permeability and T-cell secreted molecules that induce cell death, or by initiating a positive effect on cytotoxic T-cells within the tumor.[40] As the result of many oncologic studies being performed, a key conclusion is that therapeutic resistance occurs much more commonly in single-agent therapy than with multi-drug therapy.[40] Therefore, dual therapy would be a beneficial treatment for acute lymphoblastic leukemia by making leukemia cells more immunogenic while at the same time helping the cytotoxic cells of the immune system to engage these cells more efficiently, leading to an improved response and decreased likelihood of drug-resistant cells.

Dual therapy regimens have shown very promising results in other cancer studies as well. For example, Pfreundschuh et al. aimed to explore the impact of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone)-like chemotherapy regimens partnered with the monoclonal antibody rituximab for the treatment of large B-cell lymphoma.[2] For their study, 824 patients from 18 different countries were randomly assigned to either six cycles of CHOP-

like chemotherapy and rituximab or six cycles of CHOP-like chemotherapy alone. The main goal for all of the patients was event-free survival, with secondary goals of overall survival, progression-free survival, progression under therapy, and frequency of toxic effects.[2] After the median follow-up of 34 months, patients who received the combined chemotherapy-rituximab therapy had increased three year event-free survival compared with the group who were assigned only chemotherapy.[2] Before beginning treatment, the stage of lymphoma was defined by physical examination from a physician along with laboratory tests such as hemoglobin, platelets, total white blood cell count, differential WBC count, bone marrow biopsy, etc. The size of the tumor mass was assessed by local physicians and a radiologist, and then the patients were assigned to their trial, either with or without rituximab. The majority of countries participating in this trial had their patients undergo the CHOP-21 Regimen guidelines, which included 750 $mg/m^2$ cyclophosphamide, 50 $mg/m^2$ doxorubicin, and 2 mg vincristine all given intravenously on days 1, 22, 443, 64, 85, and 106; and 100 mg of prednisone were given on days 1-5. 22-26, 43-47, 64-68, 85-89, and 106-110.[2] Those who were assigned to also receive rituximab followed this schedule, along with 375 $mg/m^2$ rituximab administered intravenously on days 1, 22, 43, 64, 85, and 106 of the CHOP regimen.[2] Patients received follow-up appointments every three months for the first two years after treatment, and then every six months. Their follow-ups included the same laboratory tests administered during staging, as well as physical examination performed by the physician, and a CT of the chest and abdomen.[2] The results of which can be seen in Figure 5.[2] Furthermore, the effect of rituximab was also examined specifically in young patients. In fact, nearly twice as many (41% versus 21%) young good-prognosis patients failed after chemotherapy treatment alone rather than chemotherapy combined with rituximab.[2] Overall, patients who received rituximab and CHOP-like therapy saw a significant increase in

event-free survival, progression-free survival, and overall survival. Another study done by Sehn et al. came to the same conclusion that the addition of rituximab to CHOP-like chemotherapy resulted in significantly improved patient outcomes in the treatment of diffuse large B-cell lymphoma compared to chemotherapy treatment alone after studying a total of 292 patients.[41]

In conclusion, dual therapy treatments with monoclonal antibodies and chemotherapy have shown highly beneficial treatment outcomes when compared to treatment with chemotherapy alone. Furthermore, treatment of young patients (<20 years) also maintained the finding that the combined therapy led to increased overall survival, event-free survival, and progression-free survival. Therefore, it would be worth the cost of adding Blinatumomab into the treatment regimen for acute lymphoblastic leukemia in the pediatric population.

## Clinical Presentation and Management

### *Clinical Signs and Symptoms*

Being a cancer of the immune system, Acute Lymphoblastic Leukemia presents itself in many ways, some of them deceptive and misleading. In fact, many symptoms mimic those of the flu, but while flu symptoms eventually improve, symptoms of ALL will persist and are clear indicators to make an appointment with a physician.[42] Signs and symptoms may include bone pain, fever, frequent infections, pale skin, shortness of breath, weakness and fatigue, lumps caused by swollen lymph nodes in the neck, underarms, or groin, and possible bleeding from the gums.[42] Many of these symptoms are a result of the rapid proliferation and accumulation of undifferentiated lymphoid cells within the bone marrow, peripheral blood, and extramedullary sites.[43] Further symptoms such as anemia, thrombocytopenia, and leukopenia, all of which can be diagnosed through a physical exam and complete blood count test, can also constitute bone marrow failure within the disease.[43] Accumulation of undifferentiated cells in extramedullary sites such as the lymph nodes, spleen, and liver can lead to organ enlargement in 20% of patients.[44] Any of these signs and symptoms should be taken as a clear indicator to visit a physician.

### *Diagnostic Tests and Tools*

Typically if acute lymphoblastic leukemia or any immune system disorder is suspected, a physician will order various tests to confirm. First is a complete blood count (CBC), which measures the cell counts of several components and features of blood, including red blood cells (RBC's), white blood cells (WBC's), hemoglobin (Hgb), hematocrit, and platelets.[45] Each component of the blood plays in important role in the overall health of a patient and can give

insight into underlying medical conditions. Following the CBC, if leukemia is still suspected, the physician can order a bone marrow test. The bone marrow test consists of a bone marrow aspiration and/or biopsy. During the aspiration, a needle is inserted most commonly into the posterior iliac crest to collect a sample of bone marrow fluid.[46] If a bone marrow biopsy is also being performed, then a larger needle will be used to collect a sample of bone marrow tissue following the aspiration. After the large needle is inserted into the bone, the center of the needle is removed and the hollow needle moved deeper to capture a tiny sample of bone marrow.[47] The samples will then be sent to a lab where doctors will classify blood cells based on size, shape, and other molecular features, and hopefully determine the cause of high or low blood cell counts.[48] Diagnosis of acute lymphoblastic leukemia is typically established by the presence of 20% or more lymphoblasts in the bone marrow.[43] The samples are originally assessed via morphology, to search for cancerous cells. If the cells are found to be cancerous, the doctors will also be able to determine whether the cancer developed from B- or T-cell progenitors, thereby assisting in the potential treatment plan. The samples may subsequently undergo examination to analyze potential chromosomal changes.[49] Chromosomal analysis can include fluorescence in situ hybridization (FISH) to produce an image of the specific strands of DNA on each chromosome.[49] Following the bone marrow biopsy, a physician may choose to order an imaging test such as a computerized tomography (CT) scan, X-ray, or ultrasound to determine if the cancer has spread to other parts of the body.[48] Finally, at the time of diagnosis it is also standard for the physician to order a lumbar puncture with cerebrospinal fluid (CSF) analysis to evaluate for central nervous system involvement.[43] CNS involvement in acute lymphoblastic leukemia is a serious clinical problem that requires CNS prophylaxis. Typical chemotherapy treatments and combination therapies are not sufficient at ridding the CNS of cancer cells, and therefore

intrathecal chemotherapy and/or cranial irradiation and high-dose systemic chemotherapy are typically necessary.[50] For pediatric patients, however, cranial irradiation is highly unrecommended. Intrathecal chemotherapy is refers to treatments given through a lumbar puncture to disperse the drug throughout the spinal cord and CSF.[51] It is commonly administered to children who develop ALL in order to kill any cancer cells that may have spread to the brain and spinal cord, and prevent any others from rapidly dividing. The treatment is typically given twice within the first month and then repeated much less frequently.[51] All of these tests are typically administered to properly make an educated and informed diagnosis of leukemia.

*Treatment Plan*

Once the diagnosis is determined, the physician will then need to begin developing a treatment plan with the family. While treatment of acute lymphoblastic leukemia has significantly increased over the years and led to increased survival rates in pediatric patients, the percentage of patients who relapse and become drug resistant still receive a very dismal prognosis. Treatment of de novo patients regularly consists of 4 phases: induction, consolidation, intensification, and maintenance therapy.[49] The goal of induction therapy is to rid the blood and surrounding tissues of as many leukemia cells as possible, preferably >99%.[43,52] Following induction, consolidation and intensification therapy are administered to kill any remaining leukemia cells in order to prevent relapse.[49] Remaining cells may include those that migrate to the brain and spinal cord through the cerebrospinal fluid. In rare cases, leukemia cells can develop within the bones of the skull, and can then travel to the cerebrospinal fluid thereby affecting the central nervous system.[53] These cancerous cells are not usually affected by standard chemotherapy treatments and therefore CNS-directed remedies are necessary.

Eliminating radiation in children prevents the risk of secondary brain tumors and neuroendocrine failure, as well as other risks associated with the rapidly dividing cells children have. However, CNS-directed chemotherapy can carry with it the risk of seizures, encephalopathy, and other neurocognitive toxicities, greatly affecting function and ability of the brain.[53] The role and effect of the CNS in leukemia survival is not fully understood, and future studies in this area could be extremely beneficial in prevention of relapse ALL and treatment.

Following intensification treatment, maintenance therapy is reached to prevent leukemic blasts from redeveloping.[48] This third phase of treatment typically includes a combination of methotrexate and 6-mercaptopurine (6-MP).[54] This stage usually lasts about two years, and CNS prophylaxis treatments are typically continued as well during this time.

*Resistance to Treatment*

The resistance of some cancer cells, causing them to continue growing and dividing after treatment, leads to a devastating and dismal prognosis. Effectiveness of treatment is determined by continued tests, such as those mentioned earlier. A complete response shows that all of the cancer has disappeared and there is no further evidence of disease.[55] With a partial response, the cancer may have shrunk by a percentage but is still evident. If the cancer has neither grown nor shrunk it is referred to as stable disease, and if there is increased evidence of cancer it is defined as disease progression.[55] If cancer cells that previously appeared to be responding to treatment begin to grow, it is defined as resistance. There are several reasons the resistance may have occurred, including the appearance of clonal mutations as previously mentioned. The chemotherapy, and other factors, can mutate the cells and cause them to become resistant to the drug. As they multiply, the number of resistant cells can outnumber the cells that are sensitive to

treatment.[55] Another possible mechanism is through gene amplification from a cancer cell that leads to production of a protein that comprises the effects of the anticancer drug. Cancer cells may also use a molecule known as p-glycoprotein to pump the drug out of the cell as quickly as it is coming in.[56] These mechanisms all lead to drug resistance, and typically a new drug must be administered. In the case of resistance in relapsed cancers, the physician may recommend immunotherapy options or a hematopoietic stem cell transplant over further chemotherapy.

The road to recovery for a cancer patient can be a long battle, especially when resistance or relapse occurs, and secondary treatments become necessary. The potential to decrease the need for continued treatment and drugs, such as those used for chemotherapy, through dual therapy treatments deserves to be investigated. By introducing a combined treatment early on in the cancer fight, especially one which uses immunotherapy to encourage the patient's immune system to do the fighting, it could decrease rates of cancer cell resistance. Immunotherapy compels cancer cells to become more sensitive to chemotherapy treatment and therefore increases induced cell death.

**Research Design and Goals**

In order to determine the efficacy of dual therapy treatment with Blinatumomab and chemotherapy, experiments will need to be performed on cancerous cells. The purpose of the experiments will be to study both relapsed and primary cancer cell lines and to use those results to eventually proceed to clinical trials. Promising results would show that the dual therapy treatment leads to more efficient control and minimization of relapsed cancer cell growth, as well as decreased rates of relapsed cancer cells from primary cell lines in comparison to monotherapy treatment with either Blinatumomab or the chemotherapy drug vincristine. For these experiments, B-ALL cell lines should be used rather than murine models because they are better equipped to study the effects of drug targets on leukemia cells. While a xenogeneic mouse model is incredibly efficient at engrafting human hematopoietic and leukemia cells, and is typically the preferred model for *in vivo* research of human-derived leukemia, it will not be sufficient for research with Blinatumomab.[57,58] Xenogeneic mice are developed without a native immune system, which is key for their engraftment properties, but means that bispecific T-cell engagers (BiTE) such as Blinatumomab that are based on the immune system cannot be properly assessed.[58] Therefore, the best model to be used to study the effects of combined therapy on relapsed leukemia cells would be relapsed leukemia cells from pediatric ALL patients. Benefits of using patient-derived leukemia cell lines include an unlimited use of cell material, prolonged storage in liquid nitrogen and recoverability, long-lasting proliferation in culture, and more.[59]

After undergoing a procedure to separate out the white blood cells from red blood cells in samples of relapsed B-ALL cell lines, the collection of white blood cells will be tested for viability. Viable cells are those that are living, and are therefore the only ones used for experimentation.[59] Cell growth will be observed, and then cultures will undergo treatment with

combined vincristine and Blinatumomab to determine cytotoxicity.[59] Cell death is observed through breakdown of the cell membrane, condensation of nuclear material, and then fragmentation of the nucleus.[60] Previously, cell cytotoxic activity was measured with a Chromium[51] assay, but due to harmful effects of handling the radioactive compound, a new non-radioactive and real-time cytotoxic assay was developed.[61,62] Target-cell cytotoxicity is watched over time by imaging and live fluorescent target cells are measured in 96-well plates.[61] These images can be picked up through a fluorescent reader such as 'Cytation™ 5'.[62] By viewing the relapsed cell lines response to varying pressures, one would be able to sufficiently determine if dual therapy treatment would be worth projecting into clinical trials. If the group of cultured cells that are treated with either Blinatumomab or vincristine alone experience a greater percentage of cell death than those treated with both agents, then the conclusion could be drawn that there is a reason the agents do not work synergistically when combined. Therefore, further research would need to be done to examine the reasoning for the null results, and to thereby determine a renewed method to attack the cancerous cells. However, if the results of the experiment showed increased apoptosis in cells treated with both vincristine and Blinatumomab, then the conclusion could be made that the dual therapy treatment does in fact show promise in fighting relapsed cells, and could potentially move forward into clinical trials.

While the number of patients that recover from primary acute lymphoblastic leukemia after typical treatment regimens is ever-increasing, the issue of relapse is still one leading to a dismal prognosis. Therefore, it is vital to determine if Blinatumomab and chemotherapy dual treatment will be beneficial in decreasing rates of relapse, specifically in the pediatric population. In order to accomplish this, primary cell lines similar to those that would be used to test the combined therapy of vincristine and Blinatumomab on relapsed cells would be cultured. Primary

cell lines would be used because then the potential for this combined therapy to not only increase survival rates for relapsed patients, but to decrease rates of relapse in this patient population could be studied. Therefore, these cell lines would also be purified and cultured, and then treated in groups with either vincristine alone, Blinatumomab alone, or both agents. Cytotoxicity would be measured again as a means to determine treatment efficacy. There are many factors that have the potential to be studied within this experiment. Malignant cells that beat the drugs and continue to divide after treatment is completed would show that the development of resistant cells may be just as likely as with common treatments currently being practiced and administered. Furthermore, what if the length of time between treatment and cancer cell regrowth was significantly increased with this treatment compared to current treatments? Increasing the time between initial treatment and potential relapse means increasing the time a child can live cancer free. Or perhaps this treatment leads to an even greater percentage of cancer cells becoming resistant to chemotherapy agents, and should therefore not be administered. However, this is highly unlikely considering that the mechanism of Blinatumomab is to aid the patients' immune system to target the cancerous cell, thereby making it more susceptible to the effects of the chemotherapy without highly pressuring it to become resistant. Therefore, if the results show that the combined treatment of vincristine and Blinatumomab leads to decreased relapse percentages in the cancer cell lines, increased treatment efficacy, or even an extended amount of time between primary and secondary cancer development, then with further studies this combination could be progressed to clinical trials. Clinical trials would be able to study these processes within the human body, which is a much more complicated experimental field. There are significantly more factors that can and most likely will affect the treatment of cancerous leukemia cells. Furthermore, side effects to the patient must also be examined and

recorded. Cancer cells are incredibly adaptive, especially within the human body, therefore it is crucial to study the effect treatment has on them *in vivo*.

One of the issues being faced after treatment with Blinatumomab in acute lymphoblastic leukemia patients is that patients whose cancer cells develop clones that are $CD19^-$ will be unable to use Blinatumomab, which is known to target CD19 cell surface markers with one of its antibody arms. Therefore, for the sake of a future potential population of patients, it is crucial to determine a different cell surface marker on cancerous B cells and to develop a different monoclonal antibody other than Blinatumomab to target them. $CD19^-$ cells are extremely rare, which is why CD19 is the primary target for both acute lymphoblastic leukemia diagnosis and now immunotherapy treatment.[63] However, with the development of $CD19^-$ leukemia cells that efficiently evade Blinatumomab, it may be necessary to use other known B-cell surface markers such as CD10, CD22, or CD24 that are strongly expressed on all stages of B-cells except plasma cells.[63]

A monoclonal antibody is developed by a process of injecting a mouse with the antigen of interest, in this case CD3, and allowing its body to create antibodies for this antigen. These antibodies are then collected and washed. Following the washing stage, the cells are extracted from the liver and mixed with myeloma cells. Myeloma cells are eternal cells that when merged with typical cells, will pass on their longevity. This is key in order for more antibodies to be produced on a larger scale that fight the antigen of interest. Following, the cells will placed on a HAT medium which will intentionally kill any of the unfused myeloma cells. The cells that survive will screened by ELISA. ELISA stands for enzyme-linked immuno-absorbent assay, and is a method used to bind antibodies to soluble antigen lining the wells of a 96-well microtiter plate. This procedure tests for the binding constant of the antibodies, or in other words, it tests

their binding affinity to the antigen of choice to determine if they would be efficient and successful at marking cells for destruction. The monoclonal antibodies that are produced from this experiment will then be stripped of their disulfide bonds which unite their two halves together, and then rejoined with each other in a fashion that creates an antibody with two different heads. With these two heads the antibody is capable of targeting two different cells, one cancerous and the other normal. This will place them in a juxtaposition for attack from other components in the body. In order to determine if an antibody accurately binds to the desired antigen, an enzyme-linked secondary antibody specific for monoclonal immunoglobulins will then be used to bind to the mAb, which is then visualized by reaction with an appropriate enzyme that yields a colored product.[64] This procedure can be seen in Figure 5. The resulting antibodies can then be tested further by ELISA to determine binding affinity before placing them in cell cultures of leukemia cell clones that are $CD19^-$.

Assuming that the previous experiments were successful in increasing relapsed cancer cell death, as well as decreasing rates of relapsed cancer cells, if this experiment does not show efficient attacking and destruction of $CD19^-$ clones, then it can be concluded that there is a specific purpose behind targeting the CD19 receptor. This could mean that CD19 is crucial in the identification of cancer cells by cells of the immune system. Furthermore, if the experiment shows promising results of the cancer cell lines facing increased cell apoptosis, then it could prove that the newly generated antibody is capable of treating this specific population of patients. Furthermore, it would be interesting to continue watching the effects of this antibody on cell lines to see if new cancer cells develop that do not have the receptor that was targeted by these new antibodies. Cancer cells are incredibly versatile and adaptable, and are constantly accommodating themselves in order to survive.

**Conclusion**

Acute lymphoblastic leukemia is the number one cancer diagnosed in pediatric patients. Being derived from leukocytes, leukemia can quickly take over the patients' immune system, leading to the devasting side effects of the cancer. Without a properly functioning immune system a patient is more susceptible to infection, along with other debilitating side effects such as anemia, fatigue, and heart disease.[3] Despite significant increases in survival rates over the years, outcomes for patients who relapse are much more dismal. Typical treatment protocol for ALL consists of induction, consolidation, intensification, and maintenance therapy. Chemotherapy regimens also frequently contain the drugs vincristine and or doxorubicin, which have similar mechanisms of action in inhibiting the cancer cell cycle. Secondary treatments include immunotherapy options such as monoclonal antibodies and CAR T-cell therapy, or a hematopoietic stem cell transplant. Immunotherapy has shown the most significant benefit in patient outcomes and is therefore becoming the frontrunner in refractory cancer care. Monoclonal antibodies specifically, like Blinatumomab, have shown some of the greatest promise in fighting off cancer cells and cell clones that have become drug resistant at relapse. However, relapse remains to be one of the largest issues in treating cancer patients, and significantly decreases rates of survival, therefore methods to decrease rates of relapse are necessary. Current treatments that are being used today have been shown to increase pressure in the cancer cell environment, leading to the cell developing resistant genes to the therapy that is being administered. Therefore, a treatment that avoids this pressured environment would be incredibly beneficial to research and could lead to a significant decrease in cases of relapse.

*Efficacy Over Current Treatments*

The use of Blinatumomab in fighting ALL has recently been approved by the FDA for administration, however the dual therapy treatment of acute lymphoblastic leukemia with Blinatumomab and chemotherapy such as vincristine is hypothesized to decrease rates of relapse in the pediatric population. By combining the monoclonal antibody with the chemotherapy, the immune system will be more equipped to fight off the malignancy. As previously mentioned, monoclonal antibodies work to enhance the patients' own immune system at fighting off the cancer cells. One arm of the antibody targets the CD19 receptor on leukemia cells, while the other arm targets the CD3 receptor on the functioning white blood cells. Therefore, the white blood cells of the patient's body are more adequately prepared to perform their cytotoxic effects on the cancer cell. The added chemotherapy aids to slow down the cell cycle of the leukemia cells, increasing the efficacy of the Blinatumomab treatment. This also removes the pressure that accompanies intensive chemotherapy, thereby leading to promising results and achievements for leukemia patients in comparison to the results of current treatments. The intensive chemotherapy that is used in current treatments is incredibly harsh and non-specific, therefore leading to the debilitating side effects and potential for developed resistance. A patient undergoing chemotherapy will be constrained by this treatment for 3-5 years.[19] Children are typically not subjected to radiation due to its harmful effects on their rapidly dividing cells, thereby they require a more intensive or extended chemotherapy treatment regimen. This means more years they are missing school, missing crucial childhood experiences and relationships, and missing the opportunity to just "be a kid" because of medical appointments and treatments. This treatment has the potential to not only impact a child's medical future and health, but also their future and the experiences they deserve to have.

*Advantages to Proposed Dual Therapy Treatment*

Dual therapy treatment will be successful at both increasing survival rates of relapsed cancer patients, as well as decreasing rates of relapse in primary cancer patients. With the above proposed experiments, it can be determined whether the combined treatment of vincristine and Blinatumomab will be more effective at treating secondary leukemia, preventing relapse from primary leukemia, and also effectively treating cancers without the CD19 receptor. With the proposal of a procedure that will not only more effectively treat the cancer, but also decrease the risk of cancer cells being driven to resistance, the treatment protocol for a patient with acute lymphoblastic leukemia could be dramatically altered. Rather than undergoing years of chemotherapy treatment and maintenance, the patient would be able to recruit the help of their own immune system rather than initially significantly depleting it, which allows for the strongest response against the cancer cells. Furthermore, by decreasing the risk of relapse for the patient, it increases their quality of life and life expectancy. The survival rate of patients who relapse is significantly decreased in comparison to patients diagnosed with the primary cancer.[2] Therefore, minimizing relapse rates by preventing resistant cells will increase the overall survival and overall cancer-free survival rates of a multitude of patients. Along with increasing their lifespan, patients quality of life will also benefit from this proposed treatment. They will no longer need to spend hours upon hours going to chemotherapy treatment and living in a maintenance stage, but will receive time back with the increased efficacy of treatment. All in all, the benefits of the success of this proposed dual therapy treatment completely lie in the desire for the patient to receive the best care, and be given the best chance at a long and healthy life.

*Drawbacks and Future Research Design*

While there is incredible potential for many advantages to this combination treatment, there is also potential for problems to occur that will subsequently need to be addressed. Primarily, if there is no significant change in patient response and cancer cell cytotoxicity with the combined treatment in comparison to monotherapy treatments, then determining an alternate route, potentially with an alternate chemotherapy drug, will be necessary. The relationship between the chemotherapy and Blinatumomab would need to be further examined to determine Furthermore, there may also be potential for harmful side effects from this treatment as there are with any new procedure. While this is unexpected, due to the nature of the agents and the partnership between the monoclonal antibody and the patients cells, side effects are always present. The majority of the side effects of Blinatumomab are mild and can be easily treated, and by decreasing the intensity of the chemotherapy many of those side effects should be curbed as well. However, there are some side effects previously mentioned earlier such as cytokine release syndrome that will need to be strictly monitored. Many of these will not be recognizable until continuation into clinical trials, which would occur after success in the proposed experiments within this paper.

Furthermore, there is also the potential for increased cost of treatment in comparison to current treatments. By combining chemotherapy with the monoclonal antibody, cost will initially be remarkably higher. However, the potential for this treatment to decrease hospital visits, future chemotherapy treatments (including travel fees for the duration of consolidation and maintenance therapy), essentially outweighs the increased initial cost of the treatment. Considering that this treatment would also be applied to primary cancer patients in order to decrease their potential for relapse, it is the hope that families and insurance companies would

understand that this increased initial cost can provide relief from future costs and concerns that surface when a patient undergoes relapse.

Overall, there is significant research being done in the field of oncology, which is necessary due to the ever-changing cancer cell and its numerous methods of expression. Studying pediatric acute lymphoblastic leukemia could provide hope to many families who are fighting this seemingly never-ending battle, or for those who are fighting relapse, the seemingly undefeatable battle. There is still much work to be done, and many questions to be answered, but the progress that has been made in this field shows that each answer, however small, has purpose and can be utilized to save a life.

**APPENDIX A**

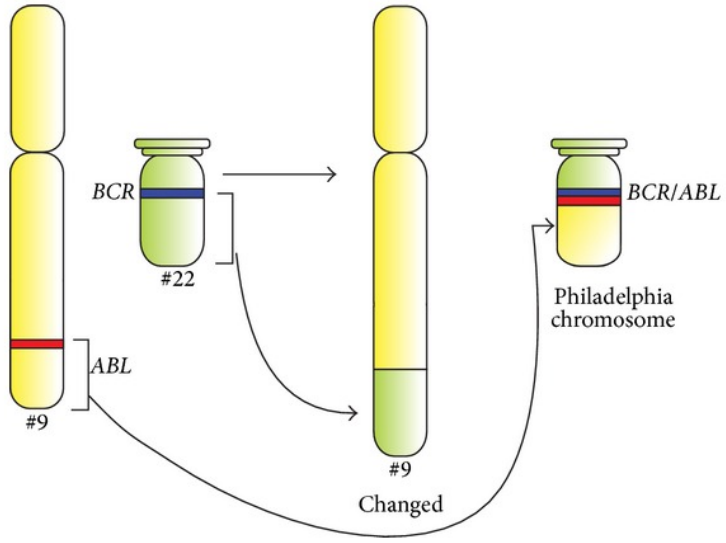


**Figure 1.** A representation of the formation of the Philadelphia chromosome from the fusion of the BCR gene and the AML gene. Both genes reside on the long arm of their respective chromosomes, 9 and 22.[7]

**APPENDIX B**

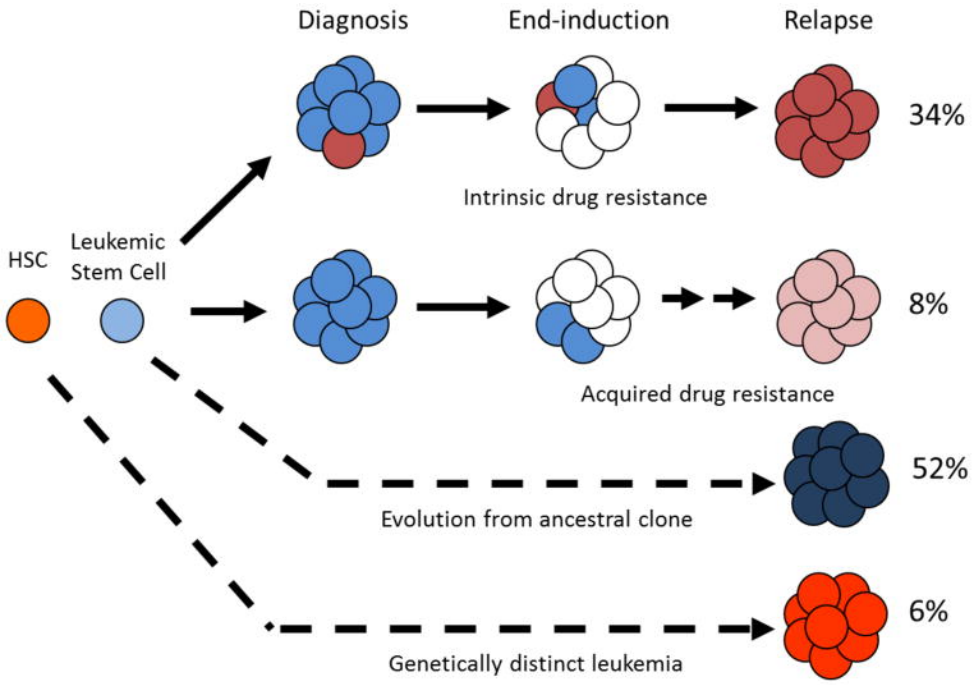


**Figure 2.** Various pathways of clonal evolution leading to drug resistant phenotype of leukemia cells. Note that approximately 94% of relapsed clones exhibit a direct relationship to the original clone present at diagnosis.[12]

**APPENDIX C**

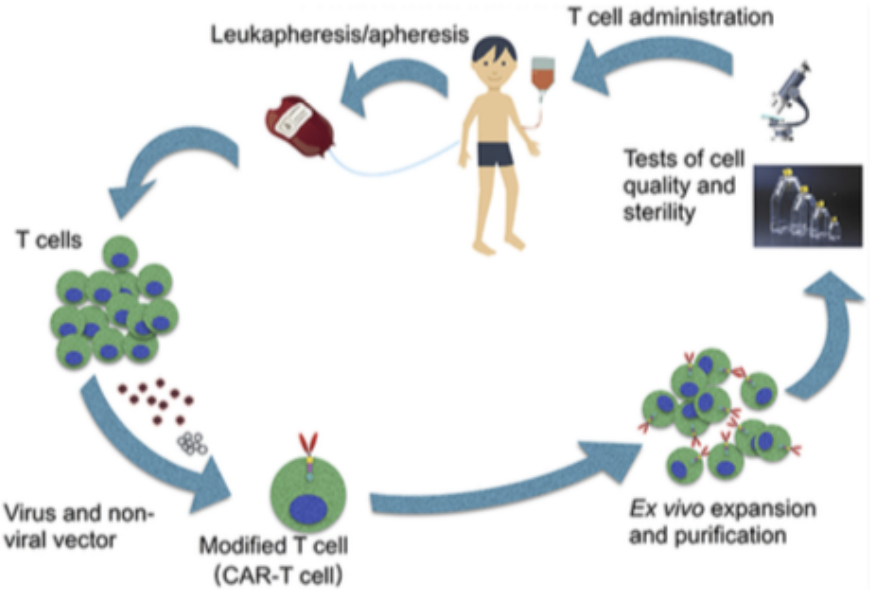


**Figure 3.** The above diagram depicts the process by which CAR-T cells are engineered and re-administered to the patient. The first step is to remove healthy leukocytes from the patient by leukapheresis/apheresis, then by introducing a viral vector into the DNA genome of the cells, they can present the CAR. Cells are then expanded and purified, then tested for quality and sterility before being administered to the patient.[30]

**APPENDIX D**

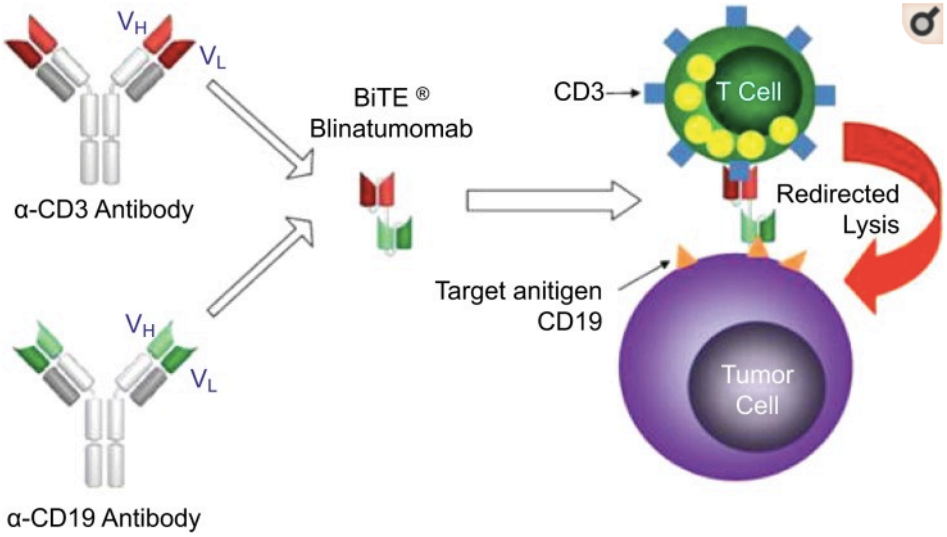


**Figure 4.** The above image depicts the mechanism through which Blinatumomab binds to CD3 and CD19 receptors to initiate an immune response.[33]

## APPENDIX E

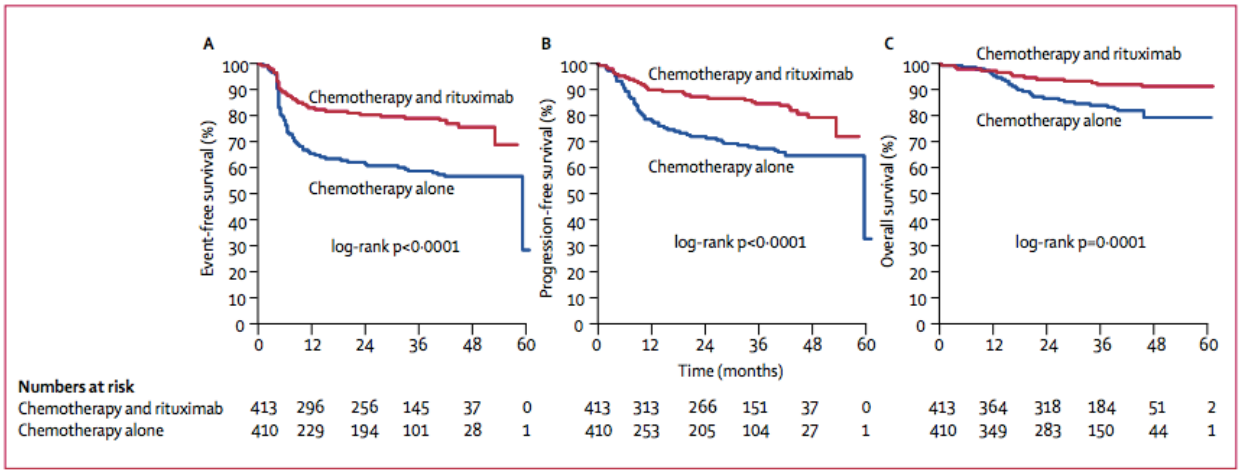


**Figure 5.** The above graphs show the comparison between patients who only received chemotherapy and those who received chemotherapy and the monoclonal antibody rituximab. Graph A depicts the rate of event-free survival, graph B shows progression-free survival, and graph C shows overall survival of the 823 patients within the trial.[38]

## APPENDIX F

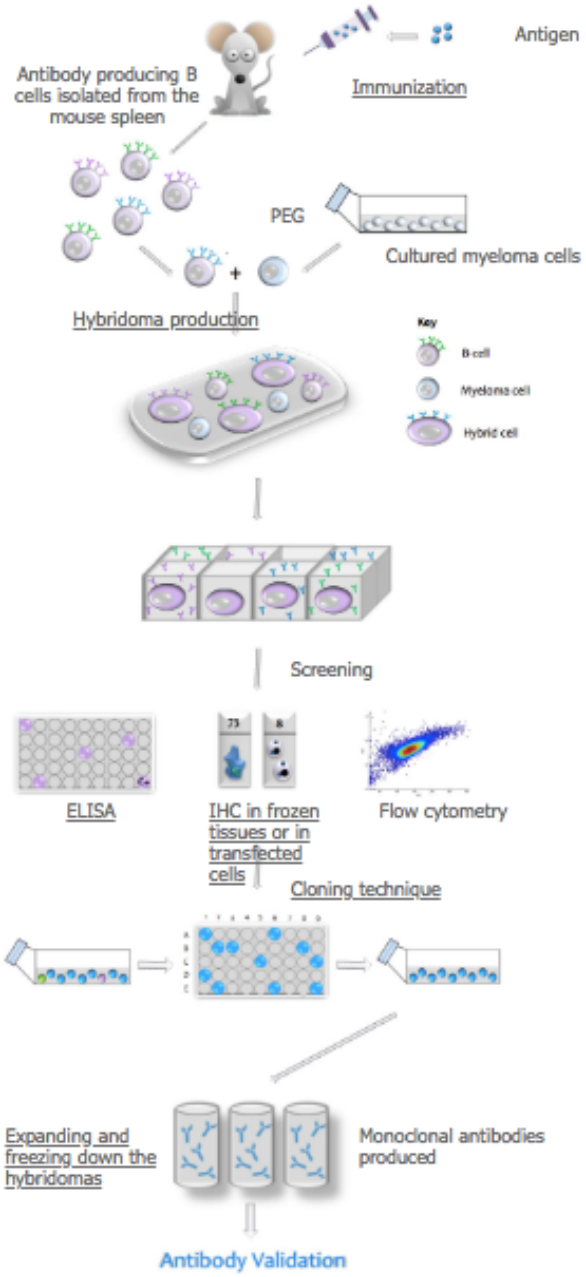


**Figure 6.** The method of monoclonal antibody production.[47]

**References**

1. Mokhtari RB, Homayouni TS, Baluch N, et al. Combination therapy in combating cancer. *Oncotarget*. 2017;8(23):38022-38043. doi:10.18632/oncotarget.16723

2. Pfreundschuh M, Trümper L, Osterborg A, et al. CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. *Lancet Oncol*. 2006;7(5):379-391. doi:10.1016/S1470-2045(06)70664-7

3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016;66(1):7-30. doi:10.3322/caac.21332

4. Whitehead TP, Metayer C, Wiemels JL, Singer AW, Miller MD. Childhood Leukemia and Primary Prevention. *Curr Probl Pediatr Adolesc Health Care*. 2016;46(10):317-352. doi:10.1016/j.cppeds.2016.08.004

5. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. *Blood Cancer J*. 2017;7(6):e577. doi:10.1038/bcj.2017.53

6. Bakalar N. History of Battle Against Leukemia. *The New York Times*. https://www.nytimes.com/2012/06/05/health/history-of-battle-against-leukemia.html. Published June 4, 2012. Accessed November 3, 2018.

7. Tasian SK, Hunger SP. Genomic Characterization of Paediatric Acute Lymphoblastic Leukaemia: an Opportunity for Precision Medicine Therapeutics. *Br J Haematol*. 2017;176(6):867-882. doi:10.1111/bjh.14474

8. Malouf C, Ottersbach K. Molecular processes involved in B cell acute lymphoblastic leukaemia. *Cell Mol Life Sci CMLS*. 2018;75(3):417-446. doi:10.1007/s00018-017-2620-z

9. Sell S. Leukemia: stem cells, maturation arrest, and differentiation therapy. *Stem Cell Rev*. 2005;1(3):197-205. doi:10.1385/SCR:1:3:197

10. Cazzaniga G, De Lorenzo P, Alten J, et al. Predictive value of minimal residual disease in Philadelphia-chromosome-positive acute lymphoblastic leukemia treated with imatinib in the European intergroup study of post-induction treatment of Philadelphia-chromosome-positive acute lymphoblastic leukemia, based on immunoglobulin/T-cell receptor and BCR/ABL1 methodologies. *Haematologica*. 2018;103(1):107-115. doi:10.3324/haematol.2017.176917

11. Sun Y, Zhao N, Wang H, et al. CT-721, a Potent Bcr-Abl Inhibitor, Exhibits Excellent In Vitro and In Vivo Efficacy in the Treatment of Chronic Myeloid Leukemia. *J Cancer*. 2017;8(14):2774-2784. doi:10.7150/jca.18731

12. Toh B, Toh B, Abastado J-P, Abastado J-P. Myeloid cells. *Oncoimmunology*. 2012;1(8):1360-1367. doi:10.4161/onci.22196

13. Schenk TM, Keyhani A, Bottcher S, et al. Multilineage involvement of Philadelphia chromosome positive acute lymphoblastic leukemia. *Leukemia*. 1998;12(5):666-674.

14. Pierro J, Hogan LE, Bhatla T, Carroll WL. New Targeted Therapies for Relapsed Pediatric Lymphoblastic Leukemia. *Expert Rev Anticancer Ther*. 2017;17(8):725-736. doi:10.1080/14737140.2017.1347507

15. Hunger SP, Lu X, Devidas M, et al. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. *J Clin Oncol Off J Am Soc Clin Oncol*. 2012;30(14):1663-1669. doi:10.1200/JCO.2011.37.8018

16. Zhang X, Wu H, Fan H, Su B, Zhang G, Dong L. Clinical characteristics and prognosis of pediatric patients with B cell acute lymphoblastic leukemia relapse. *Oncol Lett*. 2018;16(3):2929-2934. doi:10.3892/ol.2018.8974

17. Malouf C, Ottersbach K. Molecular processes involved in B cell acute lymphoblastic leukaemia. *Cell Mol Life Sci*. 2018;75(3):417-446. doi:10.1007/s00018-017-2620-z

18. Typical Treatment of Acute Lymphocytic Leukemia. https://www.cancer.org/cancer/acute-lymphocytic-leukemia/treating/typical-treatment.html. Accessed October 9, 2018.

19. Doxorubicin (Adriamycin, Rubex) Chemotherapy Drug Information. http://chemocare.com/chemotherapy/drug-info/doxorubicin.aspx. Accessed October 9, 2018.

20. Dexamethasone - Drug Information - Chemocare. http://chemocare.com/chemotherapy/drug-info/dexamethasone.aspx. Accessed October 9, 2018.

21. Vincristine - Chemotherapy Drugs - Chemocare. http://chemocare.com/chemotherapy/drug-info/Vincristine.aspx. Accessed October 9, 2018.

22. Mullighan CG, Phillips LA, Su X, et al. GENOMIC ANALYSIS OF THE CLONAL ORIGINS OF RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA. *Science*. 2008;322(5906):1377-1380. doi:10.1126/science.1164266

23. Greaves M, Maley CC. CLONAL EVOLUTION IN CANCER. *Nature*. 2012;481(7381):306-313. doi:10.1038/nature10762

24. Yang JJ, Bhojwani D, Yang W, et al. Genome-wide copy number profiling reveals molecular evolution from diagnosis to relapse in childhood acute lymphoblastic leukemia. *Blood*. 2008;112(10):4178-4183. doi:10.1182/blood-2008-06-165027

25. Bhatla T, Jones CL, Meyer JA, Vitanza NA, Raetz EA, Carroll WL. The Biology of Relapsed Acute Lymphoblastic Leukemia: Opportunities for Therapeutic Interventions. *J Pediatr Hematol Oncol*. 2014;36(6):413-418. doi:10.1097/MPH.0000000000000179

26. Copy Number Variation Analysis | CNV Array and NGS Solutions. https://www.illumina.com/techniques/popular-applications/genotyping/copy-number-variation-analysis.html. Accessed October 25, 2018.

27. Mullighan CG, Su X, Zhang J, et al. Deletion of IKZF1 and Prognosis in Acute Lymphoblastic Leukemia. *N Engl J Med*. 2009;360(5):470-480. doi:10.1056/NEJMoa0808253

28. Klumper E, Pieters R, Veerman AJ, et al. In vitro cellular drug resistance in children with relapsed/refractory acute lymphoblastic leukemia. *Blood*. 1995;86(10):3861-3868.

29. Hogan LE, Meyer JA, Yang J, et al. Integrated genomic analysis of relapsed childhood acute lymphoblastic leukemia reveals therapeutic strategies. *Blood*. 2011;118(19):5218-5226. doi:10.1182/blood-2011-04-345595

30. Naka K, Jomen Y, Ishihara K, et al. Dipeptide species regulate p38MAPK–Smad3 signalling to maintain chronic myelogenous leukaemia stem cells. *Nat Commun*. 2015;6. doi:10.1038/ncomms9039

31. Landau DA, Carter SL, Stojanov P, et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. *Cell*. 2013;152(4):714-726. doi:10.1016/j.cell.2013.01.019

32. Zhao Z, Chen Y, Francisco NM, Zhang Y, Wu M. The application of CAR-T cell therapy in hematological malignancies: advantages and challenges. *Acta Pharm Sin B*. 2018;8(4):539-551. doi:10.1016/j.apsb.2018.03.001

33. Park JH, Rivière I, Gonen M, et al. Long-Term Follow-up of CD19 CAR Therapy in Acute Lymphoblastic Leukemia. *N Engl J Med*. 2018;378(5):449-459. doi:10.1056/NEJMoa1709919

34. von Stackelberg A, Locatelli F, Zugmaier G, et al. Phase I/Phase II Study of Blinatumomab in Pediatric Patients With Relapsed/Refractory Acute Lymphoblastic Leukemia. *J Clin Oncol*. 2016;34(36):4381-4389. doi:10.1200/JCO.2016.67.3301

35. Hathaway L, Sen JM, Keng M. Impact of blinatumomab on patient outcomes in relapsed/refractory acute lymphoblastic leukemia: evidence to date. *Patient Relat Outcome Meas*. 2018;9:329-337. doi:10.2147/PROM.S149420

36. Kaplan JB, Grischenko M, Giles FJ. Blinatumomab for the treatment of acute lymphoblastic leukemia. *Invest New Drugs*. 2015;33(6):1271-1279. doi:10.1007/s10637-015-0289-4

37. Teachey DT, Rheingold SR, Maude SL, et al. Cytokine release syndrome after blinatumomab treatment related to abnormal macrophage activation and ameliorated with cytokine-directed therapy. *Blood*. 2013;121(26):5154-5157. doi:10.1182/blood-2013-02-485623

38. Liu D, Zhao J. Cytokine release syndrome: grading, modeling, and new therapy. *J Hematol OncolJ Hematol Oncol*. 2018;11. doi:10.1186/s13045-018-0653-x

39. Kantarjian H, Jabbour E. Incorporating Immunotherapy Into the Treatment Strategies of B-Cell Adult Acute Lymphoblastic Leukemia: The Role of Blinatumomab and Inotuzumab Ozogamicin. *Am Soc Clin Oncol Educ Book Am Soc Clin Oncol Annu Meet*. 2018;(38):574-578. doi:10.1200/EDBK_199505

40. Wargo JA, Reuben A, Cooper ZA, Oh KS, Sullivan RJ. Immune Effects of Chemotherapy, Radiation, and Targeted Therapy and Opportunities for Combination With Immunotherapy. *Semin Oncol*. 2015;42(4):601-616. doi:10.1053/j.seminoncol.2015.05.007

41. Sehn LH, Donaldson J, Chhanabhai M, et al. Introduction of combined CHOP plus rituximab therapy dramatically improved outcome of diffuse large B-cell lymphoma in British Columbia. *J Clin Oncol Off J Am Soc Clin Oncol*. 2005;23(22):5027-5033. doi:10.1200/JCO.2005.09.137

42. Acute lymphocytic leukemia - Symptoms and causes. Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/acute-lymphocytic-leukemia/symptoms-causes/syc-20369077. Accessed March 9, 2019.

43. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. *Blood Cancer J*. 2017;7(6):e577. doi:10.1038/bcj.2017.53

44. Alvarnas JC, Brown PA, Aoun P, et al. Acute Lymphoblastic Leukemia, Version 2.2015. *J Natl Compr Cancer Netw JNCCN*. 2015;13(10):1240-1279.

45. Complete blood count (CBC) - Mayo Clinic. https://www.mayoclinic.org/tests-procedures/complete-blood-count/about/pac-20384919. Accessed March 10, 2019.

46. Bone Marrow Tests | National Heart, Lung, and Blood Institute (NHLBI). https://www.nhlbi.nih.gov/health-topics/bone-marrow-tests. Accessed March 10, 2019.

47. Bone marrow biopsy: MedlinePlus Medical Encyclopedia. https://medlineplus.gov/ency/article/003934.htm. Accessed March 15, 2019.

48. Acute lymphocytic leukemia - Diagnosis and treatment - Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/acute-lymphocytic-leukemia/diagnosis-treatment/drc-20369083. Accessed March 10, 2019.

49. Minimal Residual Disease Evaluation in Childhood Acute Lymphoblastic Leukemia: A Clinical Evidence Review. *Ont Health Technol Assess Ser*. 2016;16(7):1-52.

50. Frishman-Levy L, Shemesh A, Bar-Sinai A, et al. Central nervous system acute lymphoblastic leukemia: role of natural killer cells. *Blood*. 2015;125(22):3420-3431. doi:10.1182/blood-2014-08-595108

51. Treatment of Children With Acute Lymphocytic Leukemia (ALL). https://www.cancer.org/cancer/leukemia-in-children/treating/children-with-all.html. Accessed March 19, 2019.

52. Scavino HF, George JN, Sears DA. Remission induction in adult acute lymphocytic leukemia. Use of vincristine and prednisone alone. *Cancer*. 1976;38(2):672-677.

53. Gossai NP, Gordon PM. The Role of the Central Nervous System Microenvironment in Pediatric Acute Lymphoblastic Leukemia. *Front Pediatr*. 2017;5. doi:10.3389/fped.2017.00090

54. Typical Treatment of Acute Lymphocytic Leukemia (ALL). https://www.cancer.org/cancer/acute-lymphocytic-leukemia/treating/typical-treatment.html. Accessed March 21, 2019.

55. How Can We Tell if Chemotherapy is Working? - What is Chemotherapy? - Chemocare. http://chemocare.com/chemotherapy/what-is-chemotherapy/how-to-tell-if-chemotherapy-is-working.aspx. Accessed March 21, 2019.

56. Chemotherapy Resistance - What is Chemotherapy? - Chemocare. http://chemocare.com/chemotherapy/what-is-chemotherapy/what-is-drug-resistance.aspx. Accessed March 21, 2019.

57. Kohnken R, Porcu P, Mishra A. Overview of the Use of Murine Models in Leukemia and Lymphoma Research. *Front Oncol*. 2017;7. doi:10.3389/fonc.2017.00022

58. Jacoby E, Chien CD, Fry TJ. Murine Models of Acute Leukemia: Important Tools in Current Pediatric Leukemia Research. *Front Oncol*. 2014;4. doi:10.3389/fonc.2014.00095

59. Drexler HG. Isolation and Culture of Leukemia Cell Lines. In: Langdon SP, ed. *Cancer Cell Culture: Methods and Protocols*. Methods in Molecular Medicine™. Totowa, NJ: Humana Press; 2004:141-155. doi:10.1385/1-59259-406-9:141

60. Ziegler U, Groscurth P. Morphological Features of Cell Death. *Physiology*. 2004;19(3):124-128. doi:10.1152/nips.01519.2004

61. Ross SL, Sherman M, McElroy PL, et al. Bispecific T cell engager (BiTE®) antibody constructs can mediate bystander tumor cell killing. *PLOS ONE*. 2017;12(8):e0183390. doi:10.1371/journal.pone.0183390

62. Fassy J, Tsalkitzi K, Salavagione E, Hamouda-Tekaya N, Braud VM. A real-time digital bio-imaging system to quantify cellular cytotoxicity as an alternative to the standard chromium-51 release assay. *Immunology*. 2017;150(4):489-494. doi:10.1111/imm.12702

63. Ghodke K, Bibi A, Rabade N, et al. CD19 negative precursor B acute lymphoblastic leukemia (B-ALL)—Immunophenotypic challenges in diagnosis and monitoring: A study of three cases. *Cytometry B Clin Cytom*. 2017;92(4):315-318. doi:10.1002/cyto.b.21373

64. Cordell J, Maestre L, Pulford K. Hybridoma Production. EuroMAbNet. https://www.euromabnet.com/protocols/hybridoma.php. Accessed November 12, 2018.

65. Gupta J, Hoque M, Zaman M, Khan RH, Saleemuddin M. A detergent-based procedure for the preparation of IgG-like bispecific antibodies in high yield. *Sci Rep*. 2016;6:39198. doi:10.1038/srep39198

66. Neves V, Aires-da-Silva F, Corte-Real S, Castanho MARB. Antibody Approaches To Treat Brain Diseases. *Trends Biotechnol*. 2016;34(1):36-48. doi:10.1016/j.tibtech.2015.10.005