Genetic Relationships and Therapeutic Options for Relapsed Acute Lymphoblastic Leukemia

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Abstract

Acute lymphoblastic leukemia (ALL) is the most common form of cancer among children and can be lethal to the adult population. Though 80% of patients with ALL reach complete remission after treatment, about 20% of those diagnosed fail to remain cancer-free. Genetic rearrangements are the hallmark of relapsed ALL, but the mechanism by which these rearrangements occur is still unclear. Recent research suggests these mutations may be detectable during initial diagnosis. If researchers are able to accurately assess the probability of relapse during diagnosis by analyzing the genome of the leukemic cells, the likelihood of administering effective therapy would increase. Providing patients with a more appropriate therapy early on may prevent relapse altogether or increase survival rates after treatment for relapsed ALL patients.

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Introduction

In 2016, there were 6,590 new cases of ALL including about 1,400 deaths (Terwilliger & Abdul-Hay, 2017). The distribution of ALL cases in the US is bimodal, in that the disease peaks in children and again in adults around the age of 50. Adults are more likely to experience complications, evidenced by only 30–40% achieving full recovery and remission. Remission is broken down into partial and complete remission groups, with the latter defined as no evidence of the disease, although cancer may still be present in the body. Partial remission is the state at which leukemic blasts have disappeared, though there may be evidence to support that they are still circulating. Increasing age correlates with decreased survival rates, which is especially poor for patients over the age of 60 due to unfavorable biology, comorbidities, and an inability to tolerate intensive chemotherapy (Terwilliger & Abdul-Hay, 2017).

ALL specifically involves diseased lymphocytes, a group of white blood cells consisting of B cells and T cells which produce antibodies and attack foreign pathogens, respectively. Precursor B-ALL is highly prevalent among children and the adult population, making up about 75% of ALL cases, whereas T-ALL is less common making up the other 25% (Raetz & Teachey, 2016). Within these large classifications, there are many different subtypes of ALL that are grouped according to similarities in genetic alterations and rearrangements of DNA sequence. Subtypes of B cell ALL are broken down into a hyperdiploid and hypodiploid subtype, depending upon the number of

chromosomes present. Hypodiploidy ALL is characterized by cancer cells containing 24-31 chromosomes. These types of ALL have poor outcomes due to the effects on tyrosine kinase and RAS signaling, which is seen in 71% of cases. Low-hypodiploidy ALL is characterized as containing 29-32 chromosomes, and the majority of these cases affect gene expression in the following pathways: p53, IKZF2, and RB1 (Terwilliger & Abdul-Hay, 2017). Also, there are six different identified subtypes depending upon the location of translocation. Two of the most prevalent subtypes within this group are translocation within the Philadelphia chromosome, which creates the BCR-ABL1 fusion gene, and translocations involving tyrosine kinases or cytokine receptors, which are known as BCR-ABL1-like ALL. The specific translocations and their downstream effectors are strong targets for therapy as they regulate cell proliferation (Terwilliger & Abdul-Hay, 2017).

Due to the genetic mutation existing in different locations of chromosomes and involving different proteins, the progression of the disease and response to treatment are variable among the subtypes. This identifies a need to further investigate the nature of the disease for each subtype, and the importance of developing drugs that effectively treat the specific disease. Chemotherapy has been widely used as an effective treatment option consisting of vincristine, corticosteroids, and the addition of anthracycline with allogeneic human stem cell transplantation (HSCT) when eligible candidates and donor standards are met (Kato & Manabe, 2018). HSCT has been found to be very effective against rare and resistant forms of cancer, but it does not provide a cure as patients still relapse after this treatment (Ceppi et al., 2016). However, researchers are still devoting

time and effort to further explore possible targets for treatment due to patients experiencing relapse of the disease after partial or complete remission (Ceppi et al., 2016). Scholars are attempting to target signaling pathways that are compromised by genetic alterations which induce the proliferation of leukemic cells in the bone marrow, blood, and extramedullary sites (Terwilliger & Abdul-Hay, 2017). Intramedullary relapse is the most common relapse site for ALL, and prognosis has a variety of ranges for these patients (Zhang et al., 2018).

Relapse, Drug Resistance, and Genetic Relationships

Though 80% of patients with ALL reach complete remission after chemotherapy/radiation treatment regimen, 20% of the pediatric population still experiences relapse of the disease. There are two conventional mechanisms by which relapse occurs: relapse may be due to a cellular clone that is resistant prior to treatment, or resistance may be induced by treatment. (Choi et al., 2007). When studying relapsed cells, researchers aim to identify the specific pathways by which they resist treatment to better prevent disease progression. Relapsed leukemic cells are found to be more related to the original leukemia rather than the secondary, or relapsed, leukemia (Bhatla et al., 2014). There are many different mutations and contributors to relapse of ALL, and many subgroups have been identified. (Bhatla et al., 2014). Research suggests the patients' bodies may also have a pre-disposition to the metabolism of drugs. Each body has a unique way by which it attempts to evade therapy and resist treatment. Relapse-specific pathways should be targeted in order to reverse the resistant nature of these cells (Tasian et al., 2015).

In a study conducted in Sydney, Australia, polymerase chain reaction (PCR) was used to detect antigen receptor gene rearrangements in immunoglobulins and T-cell receptors. In relapsed patients, PCR identified new rearrangements called relapse clones. Cohort A consisted of patients who had relapsed though minimal residual disease (MRD) results were negative while Cohort B was selected due to availability. Twenty-seven patients were analyzed, 23 with pre-B ALL and four with T-ALL. The time to first relapse ranged between 5-59 months and 12 patients relapsed while still receiving therapy. In all cases, prediction of relapse failed, and the original target was replaced by a new clonal rearrangement (Choi et al., 2007). Overall, 80% of the patients experienced an altered clonal rearrangement and 21 of the patients had one conserved marker before and after treatment. A more specific and quantifiable method, real-time quantitative PCR, RQ-PCR, was then used on eight leukemic cell samples to find that the relapse clonal marker was present at low levels upon diagnosis but was undetected. Induction chemotherapy caused the major clonal sample to decrease by 1000-fold, but the minor, or relapse clone, was reduced by 30-40-fold, suggesting that it was not only present at diagnosis, but was not reduced by therapy causing MRD. This research suggests therapy-acquired resistance is not a mechanism by which relapse occurs. Instead a subclone present at diagnosis is indicative of relapse (Choi et al., 2007).

Interestingly, quantity of the relapse clone present at diagnosis was also directly correlated to time to first relapse. For example, in four samples where the relapse clone was unable to be detected, the patients had longer remission times compared to the patients where relapse clones were detected. One patient had a subclone detected, but the

levels were too low to quantify using RQ-PCR, and this patient had the longest remission time. These findings can be used to target therapy to reduce these subclones present at low levels at diagnosis and to decrease overall relapse rates (Choi et al., 2007).

Apoptosis inhibition is a hallmark of cancer and leukemia relapse. *CXCL10* is a gene that is responsible for encoding a protein, CXCL10, involved in inflammatory response and has also been found to protect the cell from apoptosis in patients with ALL. Researchers studied this mechanism using cytarabine, which normally causes NALM-6 cells, a precursor to B-cells, to die by downregulating Bcl-2, a death inhibitory protein (Gomez et al., 2015). When the sample was treated with cytarabine in the cerebral spinal fluid, CSF, relapse sample, *CXCL10* protected the cell from the down regulation of Bcl-2, and inhibited apoptosis of NALM-6 cells. To further identify the protective nature of this chemokine, the researchers analyzed caspase activity. Normally, cytarabine activates caspases 3, 8, and 9 to induce apoptosis. However, *CXCL10* inhibited caspase activity to the state at which cleaved caspase 8 was almost undetectable in the sample (Gomez et al., 2015). Therefore, *CXCL10* may serve as a favorable target for drugs. If agents are able to decrease the expression of *CXCL10*, leukemic cells will have a higher chance of undergoing apoptosis when treated by cytarabine.

Another prominent gene, *BIRC5*, is upregulated in many ALL relapse patients. The protein product normally initiates apoptosis, but when *BIRC5* is upregulated as seen in leukemic blasts, it inhibits the activation of caspases and apoptosis, allowing continued proliferation of leukemic cells. These protein products are considered an attractive target

for treatment as researchers aim to identify agents that reverse the signature of the relapsed cancer by restoring sensitivity to the drug (Bhatla et al., 2014).

Drug resistance is the underlying property of leukemic blasts that leads to relapse of the disease and resistance can cause lower remission rates of leukemic cells or cause MRD (Pierro et al., 2017). MRD refers to a disease state in which cancer cells remain in the body after completion of treatment but are no longer detectable by current technologies. MRD has been used as the dominant predictive marker of chemoresistance and relapse. However, this disease state has failed to predict accurate outcomes in cases with unclear etiology (Choi et al., 2007) Patients who are MRD-positive only have a 25% chance of achieving 5-year remission (Gokbuget et al., 2017). HSCT improves this statistic to 44%, but only half of these patients are eligible for this treatment option due to rapid progression of relapse (Gokbuget et al., 2017).

Some scholars suggest relapse due to drug resistance may be caused by the tumor microenvironment. Tumor cells secrete and respond to chemokines, which guide leukocyte migration throughout the body and can alter anti-apoptotic pathways. This phenomenon has been observed in the central nervous system, CNS, with the survival of ALL tumor cells in organs of the CNS (Gomez et al., 2015). Out of 72 patients with B-ALL and 12 patients with T-ALL, there was an increase in chemokine receptors in the bone marrow and CSF when compared to normal tissues. In the CSF, expression of the chemokine receptor CXCL10 was increased. In addition, the CXCL10-CXCR3 complex recruited ALL cells to locations harboring lower concentrations of the antileukemic drugs, cytarabine or methotrexate, thus decreasing the efficacy of treatment. It seems that

if ALL cells express chemokines made in the blood brain barrier, BBB, due to migration, the ALL cells will compete with antileukemic drugs for their survival. Therefore, the cancer cells seem to become resistant to treatment, MRD persists, and the patient relapses (Gomez et al., 2015). Recurrent relapse of the CNS may occur due to the lack of penetration by the drug imatinib. A solution to this problem was a drug called dasatinib, which is an ABL kinase inhibitor. Dasatinib is effective by binding to these kinases and blocking their growth-promoting activities. This drug is effective due to its ability to penetrate the BBB, which was observed in a mouse model of pediatric Philadelphia (+) ALL. It has been previously documented that the most common cause of relapse was due to a mutation of the ABL kinase domain (Terwilliger & Abdul-Hay, 2017).

Many mutations and genetic correlations are associated with ALL. Genes involved in B cell development, *CDKN2A/B, PAX5, IK2F1*, and *EBF1* were all found in this mutated form in the relapsed population. There has also been relapse associated with *MSH6*, a gene coding for a protein involved in DNA mismatch repair, in attempt to resolve unwanted mutations (Bhatla et al., 2014). Other deletions are found in the glucocorticoid signaling pathway in many relapsed patients. A study conducted found that 10-14% of the relapsed population had deletions in genes *BTG1* and *TABLIX*, which are genes for a coactivator of the glucocorticoid receptor and function to degrade inhibitors of the nuclear corepressor complex (NCoR), respectively. Alterations of the NCoR are prevalent in relapsed blasts, or immature cells, and can lead to steroid resistance. Understanding this altered pathway can better identify pharmaceuticals that can be effectively metabolized by the body (Bhatla et al., 2014).

To be considered relapse-associated mutations, the mutations must either be retained from diagnosis and persist to relapse, or they must arise after treatment and correlate to relapse of the disease. Regardless of total sample size, the mutation must be present in at least two patients and absent in all patients of the non-relapse group in the individual study. A prominent biomarker in the study of relapsed ALL and progression of the disease is the transcriptional coactivator and acetyl transferase *CREBBP*. Mutations of this gene comprise 20% of all relapsed cases. (Iacobucci & Mullighan, 2017). *CREBBP* is responsible for coding the CREB binding protein, which functions to control cell division and maturation. Mutations in the functional domain of *CREBBP* suggest that it alters the function of the gene product and its function. Research also suggests that these mutations impair the regulation of glucocorticoid signaling, which will influence the cells' response to therapy (Mullighan et al., 2011).

Other prominent epigenetic regulators involved in the pathogenesis of leukemia with patients with Philadelphia negative pre-B-ALL, or Ph (-) pre-B-ALL, are SETD2, and the transcription factors *NR3C1* and PAX5. SETD2 has been previously seen to have tumor suppressor activity in breast cancer and renal cell carcinoma. Mutations here appear to form after chemotherapy and are unique to relapse. *NR3C1* codes for a transcription factor and glucocorticoid receptor. This gene activates expression to glucocorticoid response. Loss-of-function mutations in this gene are associated with chemotherapy resistance (Xiao et al., 2016). The protein PAX5, a B cell transcription factor, was studied and observed during B cell development in patients with BCP-ALL. PAX5 was overexpressed in all developmental stages when compared to that of healthy B

cell development (Good et al., 2018). Other relapse-associated genes, such as *KRAS* and *PTPN21* are found in a lower frequency of patients (Xiao et al., 2016). Mutations of these genes were found to be in the major functional domains which altered protein function. *KRAS* is an oncogene in the RAS-MAPK signaling pathway and these mutations suggest promoting tumorigenesis (Xiao et al., 2016). *PTPN21* codes for a protein tyrosine phosphatase involved in the PI3K-AKT, MAPK, and JAK-STAT pathways, all involved in regulating cell growth and proliferation (Xiao et al., 2016). Identification of the mutated genes that impair protein function can help to guide drug targeting. If mutations inhibit the normal function of these pathways, drugs may be able to target these proteins and restore their function to regulate cell growth.

Independent studies of three separate patients with Ph-negative pre-B-ALL found three separate and distinct patterns. In patient 1, the primary and relapsed tumors had eight consistent mutations found in seven genes. This pattern seems to suggest the mutations were present at diagnosis and persisted through therapy to cause relapse. In patient 2, there seemed to be a divergence in mutations from the original to the relapsed tumor (Xiao et al., 2016). The tumors shared two single nucleotide variations, SNVs, *CREBBP*, and *RGS11* mutations, but there were five additional mutations found at diagnosis that were not present at relapse. Also, there were two relapse-specific mutations in *USP54* and *NCOR2*. In patient 3, there were no mutations shared between relapse and diagnosis. Four distinct mutations were found at both stages and were unique from each other. This data suggests unique mutations to relapse which can serve as therapeutic targets, especially to prevent use of ineffective treatment (Xiao et al., 2016).

B cell Development.

B cells either originate in the bone marrow or peripheral blood. These cells are immature B cells in that when rearrangements cause proliferation to the tumor states, their development into normal cells is halted and they are unable to develop into mature B lymphocytes (Good et al., 2018). When genetic lesions are present and make DNA copy number changes, the proliferation of leukemia can be initiated (Iacobucci & Mullighan, 2017). Other research has aimed to study the development of B cells in the following stages: pre-pro-B cell, pro-B cell, and pre-B cell stages. 60 bone marrow aspirations of patients with B cell precursor ALL, or BCP-ALL, were obtained as well as samples from five non-diseased bone marrow donors. Principal component analysis (PCA) was used to compare the leukemic cells to six cellular features in normal B cell-development. PCA organizes observations of correlated variables into linear components that are not correlated (Good et al., 2018). These features were distinct as they were separated by expression of certain proteins that revealed maturity of the cell through development. The cells were assigned to the population of "best fit" as the leukemic cell does not completely resemble a normal developing B cell. They found that at all cases of study, expansion of the cells was observed in more than one stage of development. With a sample size of 54, 31% of the sample relapsed, which was higher than the literature values of 15-20%. There were three distinct patterns that were observed after PCA: an expression pattern with slight differences to the normal development, overexpression at all stages of development, or a developmentally abnormal expression pattern (Good et al., 2018). Though the proteins that were expressed in the BCP-ALL group resembled that of

a healthy B cell, the degree to which they were expressed was different. For example, PAX5 was found to be overexpressed in all developmental stages in the BCP-ALL group. Other signaling pathways such as mTOR and CREB were also elevated in this group when compared to the control sample. Therefore, overexpression of the same proteins found in healthy B cell development was correlated to overexpansion of cells and progression of the disease. This provides much insight for researchers to develop ways to restore regulation and feedback inhibition within the pathways to maintain normal development. (Good et al., 2018).

Treatment Options.

After the initial induction chemotherapy, treatment can be further administered if the disease state persists. Common types of chemotherapy include alkylating agents, antimetabolites, topoisomerase inhibitors, anti-tumor antibiotics, mitotic inhibitors, and corticosteroids. Alkylating agents target the cell cycle and damage DNA, which halts it replication and inhibits mitosis. Antimetabolites insert into DNA and RNA by acting as building blocks of the genetic code. This interference also inhibits DNA replication and cellular reproduction. Topoisomerase inhibitors impair the function of topoisomerases, which function to uncoil DNA in preparation for replication. If DNA is not unwound, it will not be replicated, and the cell cycle is unable to continue to completion. The most common type of anti-tumor drugs is a type anthracycline, which impair the function of other enzymes involved in replication. Mitotic inhibitors are a wide range of drugs that halt division in various stages of mitosis and inhibit enzymes from making the necessary proteins required to perform mitosis. Lastly, corticosteroids are hormone-like drugs that

prevent nausea and allergic reactions that are side effects of the above chemotherapy agents. Though chemotherapy is a widely used treatment for ALL, side effects exist due to the inability of the drugs to differentiate the cancer cells from the healthy cells. Therefore, there is damage to healthy tissue when treating the disease. Vincristine sulfate liposomes injection (VSLI) is a treatment option created to solve the problem of toxicity, while still maintaining efficacy. For those who have poorer responses to chemotherapy, especially adults with ALL, HSCT has been shown to successfully help patients achieve complete remission and long-term survival of the disease (Terwilliger & Abdul-Hay, 2017).

However, a percentage of patients still experience relapse of ALL after chemotherapy, radiation, and HSCT. For adults with ALL, relapse after treatment is the most common cause of death due to patient survival being only 10%. (Xiao et al., 2016). Therefore, it is necessary to develop new forms of therapy to help treat these adult patients with poor outcomes. Researchers have attempted to find mutations associated with relapse after HSCT, specifically in patients with Ph (-) B cell ALL, a cancer that has been unexplored in the past. In this study, sequencing took place at diagnosis, after complete remission, and at relapse, to compare genetic differences. There were found to be 102 sequence alterations and 25 distinct mutations in 23 genes that contributed to relapse. Many genes that had not been observed to be mutated in leukemia previously were found to be mutated in this cohort (Xiao et al., 2016). Fifty-eight Ph (-) B-ALL adult patients with normal karyotypes were studied, which had received HSCT from 2004-2008. In 2014, 28 of the patients had relapsed, with time to first relapse ranging

from 2-33 months. 30 patients did not experience relapse after being followed for 50.75 months. Of the patients that relapsed, 13 had somatic mutations of non-cancerous cells of the bone marrow, while only five had somatic mutations in the non-relapse group. Therefore, the genetic code of somatic cells must also be considered when identifying cancer mutants to discover which mutations are unique to cancer (Xiao et al., 2016).

Other Forms of Treatment.

A histone deacetylase inhibitor, vorinostat, is a probable agent that can reverse the epigenetic signature of relapse by maintaining the accessibility of chromatin to transcription factors. This agent has also been found to synergize with chemotherapy after relapse in clinical trials (Bhatla et al., 2014).

Decitabine is a demethylating agent, which was also successful in flipping the signature of relapse at diagnosis (Bhatla et al., 2014). In this study, researchers used this information to guide treatment options to counteract the behavior of relapsed cells. When treated with BEZ235, a PI3K/mTOR inhibitor, the overall abundance of active p4EBPI was reduced in pro-BII cells, but pS6 was still observed abnormal levels (Good et al., 2018). These proteins are important in the initiation of translation for protein synthesis and overall cellular growth and metabolism (Qin et al., 2016). This suggests that there are multiple ways to phosphorylate S6 and alternative signaling pathways that must be taken into consideration when designing treatment plans.

A new drug that has been studied in an attempt to improve efficacy and reduce toxicity of disease is clofarabine, which is a deoxyadenosine analog. The mechanism by which this drug improves outcome for relapsed ALL is by inhibiting ribonucleotide

reductase and DNA polymerase. Apoptosis is then initiated by the release of cytochrome c from the mitochondria. A previous phase I clinical trial of this drug was performed at the University of Texas M.D. Anderson Cancer Center with 25 patients, assessing drug efficacy by analyzing complete and partial remission. Complete remission was defined by no evidence of leukemic blasts, while partial remission was characterized by disappearance of the blasts. This means that in partial remission, though there may not be obvious blasts present, there may be *evidence* of their presence based on protein expression. In this study, five patients achieved complete remission and three achieved partial remission. In addition, the drug seemed to lack neurotoxicity. In a follow-up phase II clinical trial, 61 patients who had relapsed ALL were given clofarabine treatment of 52 mg/m^2 drug given intravenously for 2 hours daily and a total of five days (Jeha et al., 2006). This treatment was repeated every two-six weeks. 12 patients achieved complete remission accounting for 20% of the population size, and five of these patients did not have successful platelet recovery. Six patients achieved partial remission accounting for 10% of the population size. Nine of the patients were able to proceed to HSCT where seven achieved either complete or partial remission status. Thirty-five of the patients in the population were experiencing a refractory state following their most recent treatment. It is pertinent to report that 25% of the population died through the duration of this study (Jeha et al., 2006). Eight deaths were due to disease progression, two deaths were possibly drug-related, and three deaths were multifactorial, and the etiology was unclear. Infection was common among this study, many instances of which were systemic infections or sepsis due to cytokine storms. Overall, clofarabine appeared to be an

effective drug, as one third of the population achieved at least partial remission status. Also, 50% of the 12 patients that received complete remission had not responded to prior treatment. This drug shows promise in advancing ALL therapy, as it allows patients to reach an eligible state to proceed to HSCT and it proves to be active in patients with relapsed ALL. Further studies should continue to analyze clofarabine to improve its efficacy and analyze patients that are most suitable and respond appropriately to this drug (Jeha et al., 2006).

Another drug that is currently being studied is blinatumomab, a bispecific T-cell engager antibody, specific for CD19 and CD3. This drug is believed to activate T-cells which directly causes a B cell inflammatory response and ALL cell lysis. Blinatumomab was first seen to be effective in a study where 80% of the patients became MRD-negative and 60% of the patients achieved complete remission after 33 months (Terwilliger & Abdul-Hay, 2017).

In a prior phase 3 study, it showed to be an effective treatment in patients with B cell ALL and persistent MRD after chemotherapy. In the first two phases of the study, this drug achieved 43-69% MRD response, which improved by the third phase of the study. In the phase 2 study, 20 patients received Blinatumomab in four to seven-week cycles, at a dosage of 15 μg/m^2/day intravenously. 80% of the experimental population had MRD responses after treatment (Gokbuget et al., 2017). At the first follow-up at 33 months, 60% of the patients were still in remission following treatment. At the final follow up after five years, ten of the patients had continued to complete remission status. After the first cycle of Blinatumomab, nine patients were eligible and proceeded to HSCT

treatment. The ten patients who were cancer-free after the five-year follow-up included five patients who received HSCT and five patients who did not proceed with this treatment. Therefore, the drug proved to have efficacy with or without this treatment plan supplementation (Gokbuget et al., 2017). Of the six patients who had Ph-negative B-ALL, four of the patients were in complete remission after five years and none of these patients progressed to HSCT. Since half of the patients had long-term remission have treatment with blinatumomab, this drug is a possible therapeutic option for MRD-positive patients. Further studies are needed to warrant early administration of this drug rather than upon relapse and to further improve the long-term survival rates of relapsed patients (Gokbuget et al., 2017). Blinatumomab may have the ability to serve as induction therapy and therefore decrease the overall incidence of ALL relapse.

Another drug, ponatinib, is a tyrosine kinase inhibitor which resists such mutations in the BCR-ABL1 kinase domain. While research is ongoing, this drug has been approved for treatment for resistant Ph-positive ALL (Terwilliger & Abdul-Hay, 2017).

A drug that is used to treat the less common ALL of precursor T-cell lineage is called nelarabine. This drug is a T-cell specific purine nucleoside analog and has been FDA-approved. The mechanism by which nelarabine targets cancer is by accumulation of T-cells and subsequent inhibition of DNA synthesis, which ultimately causes apoptosis of the cell. A trial with this drug achieved a 31% complete remission rate for patients with T-cell ALL (Terwilliger & Abdul-Hay, 2017).

Recent research has aimed to determine if patterns of survival after relapse have changed with the development of more intensive therapies and contemporary medicine. In a study that followed a total of 9,585 patients in clinical trials from the years 1988-2002, 20.5% of these patients experienced relapse of the disease. In prior research, time to first relapse served as the strongest indicator of survival as an endpoint after treatment (Nguyen et al., 2008). For those patients relapsing in less than 18 months after first remission, a five-year survival rate is estimated at 21.0+/- 1.8%. Current treatment is suboptimal at achieving long-term survival or even short-term remission. Prior literature shows that early bone marrow relapse predicts 0-15% five-year survival rates, intermediate medullary relapse predicts a 10-40% chance of survival, and late bone marrow relapse patients have a 14-50% chance of survival (Nguyen et al., 2008). Other sites of relapse include the CNS with a 51% chance of survival and the testes with a rising 53-84% survival rate. Of the 1,961 patients who relapsed, 57.3% were bone marrow, 13.5% concurrent marrow, 20.9% CNS, 5.3% testicular, and 3.1% other extramedullary. Researchers found that the highest prevalence of relapse fell in children who were less than one year old, or older than ten. Also, males had a higher percent relapsed than females. 20% of the population who relapsed had an initial white blood count, WBC, of greater than 100,000 per μL. Ethnicity also played a role as more African American and Hispanic patients experienced relapse when compared to those of Caucasian ethnicity (Nguyen et al., 2008).

Since bone marrow relapse was the site of the majority of cases, bone marrow morphology was investigated and given a status rating depending on the percentage of

leukemic blasts existing within the marrow. M1 marrow was defined by less than 5% of circulating blasts, M2 ranged from 2-25%, and M3 was characterized by greater than 25%. Out of the 4,064 patients with M1 marrow, 16.2% relapsed at day 7 and 21.2% relapsed at day 14. There were 630 patients with M2 or M3 marrow at day 14, and 41.7% of these patients relapsed. Therefore, a slow early response to therapy is associated with event-free survival. Interestingly, patients who relapsed earlier had a lower chance of long-term survival independent of time of treatment. This suggests that although intensive treatment has been advancing with modern medicine, there was no evidence of increasing survival rates for early relapse patients (Nguyen et al., 2008). High risk patients also demonstrated an increased mortality rate independent of time. Overall, patients who were male, less than 1 year old or 10 or older, of T-cell lineage, or experiencing CNS relapse, had a higher chance of relapse throughout the time of the study. There has been improved event-free survival for patients who receive intense treatment upon initial diagnosis, however it is still unclear how to improve this for patients who fail initial treatment. This study reinforced the fact that time of relapse is a strong indicator of overall survival and it also points to the site of relapse as another prominent predictor (Nguyen et al., 2008). The challenge is to find appropriate patients at diagnosis who would benefit the most from initial, intense therapy. Further studies should be conducted to develop agents that may not normally be used in initial chemotherapy that could improve survival rates after relapse for patients with ALL (Nguyen et al., 2008).

CD19 Antigen Relapse.

Though not frequent, CD19 negative relapse is found in some patients after chemotherapy and CD19-targeted T-cell immunotherapies. The mechanism of such relapse is not well understood and explains the poor rates of survival. Though CD19 is the primary antigen expressed on B cells, it has few cell surface markers amenable to flow cytometry in the relapsed state. Therefore, loss of this antigen causes relapse of CD19 ALL to go undetected. Researchers have attempted to study B cell lineage antigens CD20, CD22, CD24, intracellular CD79a ([i]CD79a), as well as CD10, which is the most common antigen among ALL patients (Mejstrikova et al., 2017).

Blinatumomab is a new drug which has gone through many phases of study in clinical trials, specifically in the context of CD19 relapse. In past trials, 39% of the 70 pediatric patients studied achieved complete remission and 14 patients had complete MRD response to this therapy. However, 71% relapsed within 6 months and had very poor prognoses. In a study from 2017, five patients were analyzed in two separate phases which had all experienced CD19 negative relapse. The first patient achieved complete remission during cycle one and complete MRD response. But during cycle three, about three months after remission, the patient experienced relapse of the disease (Mejstrikova et al., 2017). The second patient also achieved remission in cycle one and complete MRD response. However, two weeks after induction of blinatumomab treatment, the patient experienced CD19 positive relapse. The patient continued blinatumomab and achieved a second remission in cycle two. The patient persisted to relapse two weeks after the second round of treatment but was CD19 negative. This patient died due to progression of the disease before the study was over. The third patient died as well due to relapse

about three months after induction of treatment (Mejstrikova et al., 2017). Prior to this, the patient had complete remission in cycle one, but flow cytometry showed the presence of CD19 negative blasts. Patient four experienced CD19 negative relapse in cycle four, about 45 months after remission. The fifth patient had CD19 positive blasts upon induction of therapy which changed to CD19 negative in cycle one, displaying the gain of monocytic phenotype. This data also emphasizes the importance of alternative antigens to detect this relapse due to the problem of accurate representation of disease progression when CD19 is absent as a cell surface marker. CD22 positive blasts were found to be associated with CD19 negative relapse, which may serve as an effective marker. It is important to continue monitoring patients after CD19 negative relapse in order to develop directed therapies to treat this specific type of disease progression (Mejstrikova et al., 2017).

Philadelphia (+) ALL.

Philadelphia (+) ALL has had a history of poor outcomes in regard to relapse. This leukemia is characterized by a gene expression similar to BCR-ABL Ph (+), however, it lacks the oncogene for BCR-ABLl. In this study, 46 patients with pre-B-ALL relapsed after treatment, 72% of these patients being only at medium risk according to MRD. This indicates the MRD quantitative factor may be accurate in predicting relapse in patients with Ph-like ALL. Standard and medium risk participants were treated with BFM four drug chemotherapy which consists of daunorubicin, vincristine, PEG asparaginase, and methotrexate, along with prednisolone and intrathecal methotrexate. High risk patients were given three more intense cycles of chemotherapy and stem cell

transplant therapy in addition to the above treatment. After RQ-PCR and Sanger sequencing, they found that the majority of participants had rearrangements in the gene *CRLF2* and mutations in the *JAK2* gene (Iacobucci & Mullighan, 2017).

CRLF2 codes for cytokine receptor-like factor 2, or thymic stromal derived lymphopoietin receptor. Translocation of this gene into the heavy chain of the immunoglobulin can down-regulate *CRLF2*, which directly affects the signaling pathways of JAK-STAT and Ras. Modern advancements in therapies have helped children with ALL to have better outcomes, including targeting the JAK-STAT, PI3K/mTOR, and Bcl-2 signaling pathways. Tyrosine kinase inhibitors have also shown to have success in treating Ph-like ALL (Iacobucci & Mullighan, 2017). If these therapies are successful, the cancer will no longer exist in an environment that equips the cells with the proteins needed to grow. Thus, the cells will die, and the tumor shrinks. Targeted therapies also impair the ability of the cancerous cells to undergo cell signaling with other parts of the body, inhibiting metastasis. *CRLF2* has been previously related to poor outcome due to overexpression of the gene and the downstream proteins. Of this majority, 77.7% of the participants relapsed, suggesting a clear indication that *CRLF2* rearrangements are strongly correlated to relapse risk. Knowing this information can help clinicians better treat patients that are at risk for this gene rearrangement to prevent future relapse. After these patients were followed for five years, 78% survived due to this therapy, or further stem cell transplant intervention (Heatley et al., 2017). Philadelphia-like ALL is also characterized by deletions in *IK2F1* which causes deregulation of cytokine receptors and tyrosine kinase signaling (Iacobucci & Mullighan, 2017).

Indications of Relapse and Race.

The Mexican race has been known to have higher mortality rates and incidences of relapse than any other race, as well as the highest number of patients with ALL compared to population size, than other developed countries. Mexico has not seen improvements in outcome or survival rates though chemotherapy regimens have advanced. The aim of this study was to determine factors associated with relapse in this race by using transcriptome analysis. In a study of 54 patients with ALL, bone marrow samples were obtained, and the patients were followed from 2014-2017 (Nunez-Enriquez et al., 2016). Eleven patients relapsed before the first 18 months and half were considered high risk at diagnosis. Interestingly, less than 20% of these patients had common genetic rearrangements secondary to ALL. Only one third of ALL patients from developed countries are considered high risk and about 32% of these individuals exhibit the common gene rearrangements in their genome. Over half of the population that relapsed were considered standard risk, and many of these individuals relapsed during treatment (Nunez-Enriquez et al., 2016).

Very early relapse was found to be common within this population, and time of first relapse has been known to be a significant indicator of survival as an endpoint. The 11 patients that relapsed achieved this stage in an average of 10.6 months and five of these patients died after isolated bone marrow relapse. It has been previously understood that time to first relapse and relapse site are large predictors of survival, and this is especially evident in the Mexican ALL population (Nunez-Enriquez et al., 2016).

Many genes were found to be up- and down-regulated which play roles in B and T cell activation, EGF and FGF signaling, and induction of apoptosis pathways. The highest abnormally expressed gene was *BLVRB* and previous data suggests that this gene is associated with prednisolone therapy resistance, a hallmark of relapse. *TMODI* was another gene shown to be overexpressed in this cohort. This gene codes for an erythrocyte membrane protein and when it is mutated, red blood cells' membranes are altered, leading to proliferation of cancer cells (Nunez-Enriquez et al., 2016). The PAX5 pathway was overexpressed, however BLNK, an adapter protein in BCR signaling for apoptosis was down regulated. Mutations of this gene completely block B cell development, leading to pre-B cell ALL. A knockout mutation was performed with mice to confirm these findings and the results concluded that the mutation inhibited B cell development

EBF1 is also a gene which is dependent on PAX5 and deletions are commonly found in *EBF1* to be associated with leukemogenesis in Down Syndrome patients. PAX5 is also a regulator of FLT3, and its increased expression leads to decreased B cell lymphopoiesis. Loss of PAX5 leads to altered lymphoid and myeloid development leading to abnormal expression of myeloperoxidase, or MPO, due to the absence of other myeloid markers. Usually ALL blasts are negative for MPO, but it has been detected in this cohort and may serve as a biomarker for MRD (Nunez-Enriquez et al., 2016).

Risk Stratification & Predictive Markers of Relapse

Identifying disease risk factors and prognosis is essential when considering treatment plans for patients with ALL. When considering prognosis and risk, MRD has

been very effective in predicting success of induction chemotherapy. This is also a way to assign patients to high, intermediate, and standard-risk statuses and to group treatment accordingly (Terwilliger & Abdul-Hay, 2017). As age increases, the risk of ALL relapse also increases (Iacobucci & Mullighan, 2017). Age and white blood cell count at diagnosis were previously seen as the main factors affecting long term outcome. However, with advancement in research and technology, the cytology and genetic factors of leukemic cells have had a much greater effect on accurately predicting outcomes and appropriate treatment options. High risk patients such as Ph (+) have been previously predicted to have a 10% chance of one-year survival. However, with recent research shifting toward cytogenetic factors, scholars have been able to develop tyrosine kinase inhibitors that have been very effective in treating ALL. Other high-risk subsets consist of Ph-like ALL in which patients do not respond well to the first round of chemotherapy and are likely to relapse due to high MRD (Terwilliger & Abdul-Hay, 2017).

Studying the expression of proteins on the leukemic cells and the progression of the disease at the cellular level can also provide insight into predictive markers for relapse. Though theories exist that cancer cells become resistant through the induction of therapy, it has also been widely accepted that resistance is present at the time of diagnosis and persists through therapy, leading to relapse (Fuster, 2014). Since such resistant cells are rare, they must be studied on a single-cell basis in order to analyze the cell signaling pathways that are associated with relapse at diagnosis. There have been prior risk-prediction methods to study the rare resistant cells and the phenotypic differences between non-relapse cells, as well as biochemistry to define the mechanism by which

these resistant cells evade treatment. However, current methods are not completely accurate at successfully identifying relapse (Good et al., 2018). Researchers have aimed to develop a model that would not only predict outcome of disease but identify cellular features that are indicative of relapse and can be a better predictor of relapse risk than current models. The name of this model was Developmentally Dependent Predictor of Relapse, or DDPR. Using this method, the researchers found that pro-BII cells had an increase in basal activation of the mTOR pathway and pre-BI cells exhibited a decrease in response to stimulation in the pre-BCR pathway. This model was successful at determining relapse-free survival after single cell development classification. DDPR was able to identify cellular features indicative of risk for relapse, and its guided treatment options available to such patients. These features were not only found at the time of relapse, in fact, they were evident at diagnosis and persisted at relapse to an overexpressed state. Regarding cellular behavior, the strength of signaling in the mTOR pathway was enhanced at relapse, allowing the proliferation of the tumor (Good et al., 2018).

There were multiple benefits to this approach of studying the leukemic blasts on a single-cell basis. The researchers found features and identifiable cellular behaviors that related to clinical outcome. Also, they discovered insight into the mechanisms by which the tumor state persisted, compared to that of normal B cell development. The most prominent stage of development for transition from a benign to malignant state was the pre-pro-B cell to pre-BI cell state. Overall, this study added to the literature and

knowledge of risk for relapse and devised a model to accurately predict relapse according to cellular features (Good et al., 2018).

Conclusion.

Fully understanding the multifactorial hallmarks of ALL is the best way to effectively treat this cancer. High risk patients include those that are Ph (+), exhibit high WBC counts at diagnosis, harbor CNS disease, contain high-risk genetic rearrangements, and are hypodiploid. If these factors are first taken into consideration the disease can be better treated initially and prevent long-term use of toxic drugs. HSCT shows much promise for long term survival in qualified patients, especially those who are high-risk. Modern clinical trials of many new drugs have improved prognosis and survival for relapsed patients following treatment when compared to that of chemotherapy. Since each ALL case is unique due to patient demographic, family history, and genetic alterations, it is very unlikely that a single drug or agent will have the capacity to be effective in all ALL patients. Current researchers strive to continue to advance treatment options by exploring new agents and targeted therapy.

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