Analysis of Current and Potential Treatments for Chronic Lymphocytic Leukemia

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Abstract

Chronic Lymphocytic Leukemia (CLL) is one of the most common forms of adult leukemia in North Americans. However, it has been shown through the analysis of clinical studies and synopses of medical research that no current treatment indefinitely cures CLL or prolongs the life of CLL patients. Current CLL therapies are either symptomatic treatments (chemotherapy and immunotherapy) that do not improve complete remission rates or effective treatments that target the actual cancer but have excessive morbidity and toxicity rates (stem cell transplants). Conventional CLL treatments are inadequate according to the results of clinical trials and research of field experts. Potential fields of research (nanotechnology, gene therapy and phytotherapy) that show promise as CLL therapies according to the studies of researchers and scientists should be further examined. If a cure for CLL is to be found, the focus of treatment needs to move away from symptomatic relief toward the identification and remedy of the cause of the cancer.

Analysis of Current and Potential Treatment for Chronic Lymphocytic Leukemia

Background

Chronic Lymphocytic Leukemia (CLL) is one of the most prevalent blood cancers among North American adults (Yee & O'Brien, 2006). It is a leukemia that originates in the bone marrow with the lymphoid line of cell development. Unlike cancers typified by amassed tissue tumors (carcinomas, sarcomas, lymphomas), leukemias are accumulations of single, hematopoietic cells in the bloodstream or lymph. (Kindt, Goldsby & Osborne, 2007). Generic cells (hematopoietic stem cells) mature into either myeloid or lymphoid ancestral cells from which all other specialized cells arise. Leukemias occur when there is a malfunction in the lymphoid lineage from which natural killer cells, T cells, B cells and dendritic cells are derived.

CLL lymphocytes develop from normal B lymphocytes (Chiorazzi, Rai, & Ferrarini, 2005). When CLL is present, mutated lymphocytes that cannot perform their disease-fighting role reproduce uncontrollably. They populate the bone marrow then build up in the bloodstream, hindering the production of other cells. The numerous lymphocytes also cause the spleen and lymph nodes to enlarge.

Currently there is no cure for CLL (Hamblin, 2001). Generally, CLL is treated with chemotherapy which has side effects that can be as devastating as the disease itself.

Healthy Immune System

There are two arms to the human immune system: innate (natural, inherited and nonspecific) and adaptive responses (Kindt et al., 2007). CLL is thought to have its origins in the adaptive component of the immune system which consists of a highly

specific detection and removal of foreign invaders (Chiorazzi et al., 2005). Adaptive immunity is characterized by an ability to recognize and remember these foreign antigens. The adaptive immune system also discriminates between foreign and body cells and between varieties of antigens.

The main agents of adaptive immunity are white blood cell lymphocytes. Normally, generic stem cells propagate along the lymphoid line of development to become one of three types of specialized lymphocytes:

Natural killer (NK) cells are nonspecific immune cells that belong to the natural innate immune system (Coleman, 2010; Kindt et al., 2007). They lack specialized surface molecules that would allow for antigen-specific responses. Instead, NK cells detect general anomalies associated with infection like abnormal numbers of a type of surface proteins (MHC, major histocompatability complex). Defective cells are then eliminated by the natural killer cell attaching to its surface antibodies and releasing membrane-compromising substances.

The other two types of lymphocytes in the lymphoid lineage are part of the highly specific adaptive immune system, with cell surface proteins that enable antigen-specific recognition and response (Coleman, 2010; Kindt et al., 2007). T cells recognize specific antigens only when their matching antigen is attached to another cell's MHC (cell-surface proteins that present antigen). There are three types of T cells which become either active or memory T cells upon encountering their antigen in conjunction with an MHC. In activated form, T helper (T_H) cells secrete cytokine signalling proteins to induce response of other immune agents or function as long-lasting memory cells. Upon antigen exposure, Cytotoxic T (T_C) cells become memory cells or cytotoxic T lymphocytes

(CTLs) which eliminate cells presenting antigen on their MHC. T regulatory (T_{reg}) cells down-regulate the immune system when activated, playing a repressive role.

The other type of lymphocyte, B cells, produce proteins specific to a certain foreign antigen and present these antibody proteins on their cell surface (Coleman, 2010; Kindt et al., 2007). When the matching antigen binds to its membrane antibody, a B cell undergoes heightened reproduction. The progeny clones become specialized plasma or memory B cells with the same antigen specificity as the parent B cell. Plasma cells create a secreteable form of the original B cell antibody. Memory B cells are long-lasting clones involved in immune response if the same antigen is encountered again. B cells are the lymphocytes that are central to CLL.

The site of B cell origination and maturation is the bone marrow (Chiorazzi et al., 2005). Here, genetic rearrangement takes place, yielding B cells with different membrane antibodies that function as antigen receptors. These B cell receptors are made up of two polypeptide chains (heavy and light) with constant regions and variable antigen-binding regions that are varied by this genetic process. The result is a population of B cells, each with its own set of antibody surface receptors, specific for binding different foreign antigens.

Encounters between these mature B cells and their antigens take place within the lymph system (Coleman, 2010; Nester, Anderson & Roberts, Jr., 2007). Fluid full of mature lymphocytes (lymph) circulates through lymphatic vessels, tissues, organs like the spleen, and lymph nodes. Lymph nodes act as filters, bringing invading antigens or malfunctioning host cells and B cells in contact in order for binding to stimulate B cell

activation and consequent immune response. CLL is thought to develop from antigen-activated B cells (Chiorazzi et al., 2005).

B cell activation requires both the binding of the B cell receptor's specific antigen and an authorization signal from an active T_H (Nester et al., 2007). When an antigen binds to its matching B cell receptor, it gets taken into the B cell and broken down. The B cell then displays pieces of the antigen on its cell surface MHC. If a T_H receptor recognizes the antigen, it will signal the presenting B cell to proceed with production of a population of clone B cells (proliferation). During this reproduction, DNA undergoes genetic rearrangements that affect the antigen-binding of the clones' antibodies (Kindt et al., 2007). Clones with reduced antigen binding are apoptotically eliminated while those with high affinity antibodies can become specialized plasma cells or Memory B cells.

The short-lived plasma cells mass produce and release free-floating antibodies which recognize the same antigen as the membrane-bound antibody of the parent B cell (Nester et al., 2007; Kindt et al., 2007). Plasma-secreted antibodies bind the encountered antigen and aid agents of the immune system like phagocytes, and cytotoxic NK cells to perform their roles in antigen destruction and removal.

Newly-formed plasma cells all emit IgM class serum antibodies (Kindt et al., 2007). However, many plasma cells undergo genetic rearrangements in the constant regions of their antibodies in order to elicit a location-appropriate immune response. A switch in the type and function of antibody produced occurs while maintaining the variable region and specificity of binding for the original antigen stimulator (Kindt et al., 2007; Nester et al., 2007). For example, tissue membrane plasma cells often switch from

producing serum IgM to secreting more appropriate mucosal IgA antibody with the same antigen specificity.

Memory B cells (longer-lived B cell clones) develop after differentiation and any antibody class switching has occurred. These B cell clones are involved upon consecutive exposure to an antigen and enable a more rapid, effective secondary immune response.

CLL Immune System

Phenotype and development. In its most basic form, cancer is characterized by abnormal cells that are able to escape immune screening, multiply and form groups (Coleman, 2010). CLL is no exception. The diagnostic phenotype of CLL is the buildup of a population of mature B cells with a certain class of surface markers (CD5+) and low levels of IgM and IgD surface antibodies (Dighiero, et al., 1998; Keating et al., 2003). A small number of these CLL cells are at a replicative stage while most are stopped at a non-reproductive stage in the cell cycle. CLL cells have proteins that increase anti-apoptosis mechanisms and decreased levels of apoptotic proteins (BCL-2 and BCL-X , respectively).

There are many factors that may give clues as to the nature of the origin of CLL. CLL is more frequent in the elderly and two times more frequent in men (U.S. News & World Report, 2008). Those of Asian descent are at a much lower risk for the disease and there is a familial tie. CLL is not considered a heritable disease but the predisposition for CLL is thought to be inherited. However, it has yet to be determined which of these factors are relevant to the cause of CLL.

Fluorescence in situ hybridization has detected genetic anomalies in a majority of CLL patients but a single gene or genes that undisputedly cause CLL have yet to be found (American Cancer Society, 2007). CLL patients often received mutations from a parent that increased their risk of developing CLL but did not directly cause the disease. Genetic predisposition and the resulting population of precursor CLL B cells are thought to play a role in the development of CLL (Montserrat & Moreno, 2008). However, precursor monoclonal B cells are present in a percentage of the healthy population so further mutation events that trigger CLL must occur after birth for the disease to develop.

The trigger event for CLL development is thought to begin with foreign or self antigen stimulation and subsequent clonal expansion of a genetically predisposed B cell (Chiorazzi et al., 2005; Lanasa, 2010). A number of CLL patients show similarities in antigen-receptors on their CLL B cells, indicating that a certain group of antigens may be responsible for the propagation of pre-CLL cells and development into CLL. CLL cells have multiple antigen binding sites so the trigger event could involve foreign antigens, self antigens or combination stimulation.

Antigen activation followed by random mutations that provide survival advantages and continual antigen encounter can select for a pre-CLL B cell (Montserrat & Moreno, 2008). A majority of these cells are stopped by cell cycle checkpoints for defects but a small percentage makes it through to reproduce without the mechanisms necessary for apoptosis. If genetic mutations and other external and cellular events provide survival advantages, a CLL cell that avoids apoptotic immune response can propagate.

A variety of cell components are associated with CLL cell immune system avoidance and survival (Chiorazzi et al., 2005). CLL cells act both offensively and defensively against apoptosis. The presence of BCL-2 proteins on CLL cells increases anti-apoptotic mechanisms and decreased levels of apoptotic BCL-X reduce programmed cell death mechanisms. Traditionally, CLL has been attributed only to lack of apoptosis since most CLL cells in circulation are not at the replicative stage in the cell cycle (Lanasa, 2010). However, a small number of these CLL cells are undergoing replication and other characteristics like CD markers associated with activation suggest that CLL cells may have increased cloning abilities in addition to decreased apoptosis. Without regular controlled cell death, CLL cells are able to avoid normal eliminating immune responses and reproduce uncontrollably.

A number of external factors also promote the survival and propagation of CLL B cells (Montserrat & Moreno, 2008). T cell levels in CLL patients are significantly increased in number but decreased in diversity. T-cell populations are shifted toward a decrease in immune response. T cells associated with immune suppression (T_{reg}) are enabled to survive by the same anti-apoptotic BCL-2 protein as CLL cells. This results in longer surviving suppressive T_{reg} cells which may partially explain why CLL cells are able to escape the immune system.

CLL cells are also protected by a microenvironment of cells and substances that provide the necessary signals, nutrients and processes for survival (Keating et al., 2003). Included in these supportive cells are CLL blood nurselike cells which attach and congregate CLL cells, shielding them from therapeutic drugs. Mesenchymal stromal cells provide a vascular system to the CLL cells (Lanasa, 2010). Cell signal cytokines

and chemokines that serve as clonal expansion promoters and are transmitted by direct contact, signal delivery and antigen presentation by the cells of the microenvironment.

Symptoms. When CLL is present, mutated B cells that cannot perform their disease-fighting role reproduce uncontrollably (Mayo Clinic, 2010). CLL cells populate the bone marrow then build up in the bloodstream and lymph system. This results in a variety of health complications.

Lymph nodes and spleen enlarge from CLL cell buildup. Accumulation in the bone marrow hinders the production of other cells and causes serious health problems. Decreased red blood cell production leads to anemia and associated symptoms. Low platelet levels result in increased bruising, bleeding and hemorrhage. Low white blood cell levels make CLL patients critically vulnerable to infection.

As previously mentioned, high levels of suppressive T cells are characteristic of CLL. Consequently, CLL patients face a much greater possibility of infection (Coleman, 2010). T-cells which normally help B-cells produce antibodies can malfunction, causing the immune system to attack the body rather than invading cells (autoimmunity).

CLL is a cancer that is attributed to the buildup of a specific type of mature B lymphocytes in the blood, bone marrow and lymphatic system. The leukemia is thought to develop from normal, mature B cells as a result of a combination of events and factors. Genetic predisposition, antigenic encounter and cellular and environmental survival-promoting factors are thought to lead to the survival and growth of CLL B cell populations. While much information on CLL characteristics, symptoms and progression is available, scientists have yet to discover the root cause that initiates development of the disease.

Current Therapies

Chemotherapy

Chemotherapy is a leukemia treatment that uses drugs to stop cell division (National, 2007). There are different types of chemotherapy, each with its own target and goal. Some aim to wipe out the cancer while others try to stop cancer from progressing while treating any symptoms. (Rai, Keating, Larson & Connor, 2005)

The main focus of CLL chemotherapy has tended to be symptomatic relief with the lowest risk of health complications from treatment since there has not yet been a chemotherapy treatment demonstrated to result in longer life of patients.

Complete remission (CR) is a term used to describe a state of cancer-free blood and bone marrow as well as normalized blood counts (Wierda, 2006). This state is associated with longer survival than partial or no remission in response to therapy. The goal of chemotherapy in regards to CLL is to obtain high rates of CR in patients and extend remission as long as possible in hopes that overall survival (OS) rates will also increase.

Alkylating agents (Chlorambucil). Alkylating agents result in some symptomatic improvement, but not all symptoms disappear completely and survival is not prolonged. Comparative studies affirm this information with partial response rates as high as 72% but chances of complete remission as low as 4% (Palma et al., 2006).

Alkylating agents are a group of drugs that hinder the process of cell replication (Encarta World English Dictionary, 2007). They modify DNA by inserting an alkyl hydrocarbon group. When this group is present, the double helix DNA cannot unwind.

As a result, the targeted cells cannot reproduce and eventually die. (American, 2007; Hamilton, 2005).

Chlorambucil, which is the most common alkylating drug for CLL, has been shown to have an over 50% partial response rate but no significant increase in complete cure rates (Canadian Cancer Society, 2006).

Unfortunately, the side effects of alkylating therapy overshadow the potential for symptomatic relief (American, 2007; Hamilton, 2005). Most alkylating agents are derivatives of poisonous substances such as mustard gas and are understandably associated with detrimental effects. The first issue with the drugs is the non-discriminatory nature of their treatment. Alkylating agents target all fast growing cells, not just cancerous B lymphocytes. This leads to lowered blood cell numbers overall (red, white, and platelets) which increases risks of infection, bleeding, bruising, anemia and fatigue in patients. Alkylating drugs also attack intestinal cells causing queasiness, appetite loss and vomiting. Other side effects include hair loss and mouth sores.

Alkylating chemotherapy also increases risks of secondary cancers developing (Abbott, 2005). One clinical study treated 1384 CLL patients with alkylating chemotherapy. The study showed a 250% increase in risk of patients developing a second, more aggressive cancer (acute myeloid leukemia).

Alkylating chemotherapy is associated with extensive side effects including increased risks of infection and development of additional malignancies which must be weighed against the potential for partial symptomatic improvement. With alkylating treatment of CLL "the quality of response is low (CR [complete response] 0%-31%) and the duration of responses usually short (2-18 months)" (Yee & O'Brien, 2006, p. 1114).

While partial relief of some CLL symptoms may be obtained with alkylating therapy, it comes at a high risk of serious health complications and lowered quality of life.

Purine nucleoside analogs (Fludarabine, Cladribine). Purine analogs are a newer form of chemotherapy drugs that work in a slightly different way with different results from alkylator therapy (Encarta, 2007). Purine analogs act as inhibitors to DNA replication. They either replace or inhibit CLL enzymes such as DNA polymerase which is essential to DNA replication (Palma et al., 2006). Without such enzymes DNA reproduction is halted and apoptosis is stimulated, killing off cells. The main purine analogs used in CLL treatment are Fludarabine and Cladribine.

Studies show that compared to alkylator therapies, Fludarabine and Cladribine have higher CR rates, longer periods of CLL dormancy and greater symptomatic response. However, purine analogs show no significant improvement in overall survival rates (Dighiero et al., 1998; Eichhorst et al., 2009).

One study tested alkylator chlorambucil and purine nucleoside Fludarabine as monotherapies and also in combination (Wierda, 2006; Rai, Peterson & Appelbaum, 2000). The combination therapy proved too toxic and could not be continued, but Fludarabine yielded CR rates more than 15% higher than Chlorambucil with remissions usually lasting 25 months. However, overall survival rates were not increased in comparison to alkylator chemotherapy trials (Rai et al., 2000; Weirda, 2006).

Also, purine analogs demonstrate a significantly greater number of side effects than alkylator therapy (Palma et al., 2006). Fludarabine does not specifically target cancer cells so it also kills T-cells causing the immune system to become severely suppressed, comparable to that of an HIV/AIDS patient. The immune system can remain

at this vulnerable state for years. With low T-cell levels, patients can develop Richter's Syndrome which is a more aggressive cancer that affects major areas such as the brain and becomes lethal after 5-8 months. Post-treatment immunosuppression also leads to high rates of infection by a variety of harmful viruses, bacteria and fungi (Hamblin, 2001).

Fludarabine may also cause CLL to develop into a less treatable form of the disease (Hamblin, 2001). CLL cells without a certain protein (p53) are immune to Fludarabine. Therefore, when Fludarabine wipes out CLL cells, resistant CLL cells are left behind that can only be treated with harsher therapies that have increased risks of infection and other severe side effects.

The risks of getting deadly hemolytic anemia (red blood cell shortage), slowed blood cell production, painful shingles herpes and other serious problems are high with Fludarabine (Hamblin, 2005; Abbott, 2005).

Other purine analogs like Cladribine and Pentostatin show similar or poorer results (Kay, Hamblin, Jelinek, Dewald, Byrd, Farag, Lucas & Lin, 2002). Purine analogue chemotherapy has a long list of side effects including bone marrow suppression, severely low lymphocyte, platelet and neutrophil levels, immunosuppresion associated with secondary malignancies and high rates of infection by a variety of opportunistic pathogens (Hamblin, 2001).

With increased risk of immuno- and myelosuppression, infection, anemia, secondary cancers and CLL mutations, alkylating agents provide temporary symptomatic relief but also increase chances of detrimental health problems and ultimately, shortened

life (Byrd, 2006). Purine analog therapy may yield higher response rates than alkylator therapy but it also shows increased severity and occurrence of negative side effects.

It is clear that chemotherapy adds more health problems to the already heavy load CLL patients bear. Chemotherapy can provide temporary symptomatic relief but no increase in overall survival. It increases chances of developing serious health complications that can decrease survival length of patients. As a result it can be concluded that treatment based on chemotherapy is not the source of a cure for CLL (Byrd, 2006).

Immunotherapy-Monoclonal antibodies (Alemtuzumab, Rituximab). The main immunotherapy used to treat CLL is synthetic monoclonal antibodies (Kindt et al., 2007). Monoclonal antibodies (MABs) are antibodies specific for a single B cell clone that has responded to one particular binding site (epitope) of an antigen. MABs are synthesized by the replication of an artificial cell in vitro and employed in order to boost the immune response of a CLL patient (National, 2007).

In the case of CLL, MABs are made to identify and lock onto CLL cells or cells of specific substances that allow CLL cells to survive (National, 2007). The immune system is then able to target and eliminate these cells. Locked-on MABs can also work to block the growth, reproduction and spread of CLL cells. Another possible method is for MABs to deliver toxic compounds exclusively and directly to CLL cells. MABs are thought to induce cell death of CLL by direct drug transport, immune response stimulation, limiting supply of needed substances, or blocking reproduction.

The two major MABs in CLL treatment are Alemtuzumab and Rituximab. Alemtuzumab is an antibody against the CD52 surface proteins found on almost all

mature lymphocytes (Flynn & Byrd, 2000; Kay et al., 2002; Hale et al., 1983). This antibody locks on to B cells and facilitates cell lysis, wiping out all CLL cells while leaving immature stem cells unharmed. In vitro tests have shown Alemtuzumab is associated with nearly complete elimination of lymphocytes.

Rituximab is another MAB against the CD20 surface markers found on precursor and mature B cells (Cataland, Lucas & Byrd, 2005). Rituxmab attaches to CLL B cells which recruits immune agents involved in cell lysis or induces B cell apoptosis (National, 2007; Shaw, Quan & Totoritis, 2003).

Overall, MABs are not successful as a monotherapy. In a clinical study, Alemtuzumab was injected into 38 CLL patients over a period of 18 weeks. Alemtuzumab was found to have a 19% Complete Response rate (Lundin et al., 2002).

Rituximab was shown to have even lower CR rates (4%) when tested as a monotherapy in a trial of 44 untreated CLL patients (Hainsworth et al., 2003).

In addition to the low CR rates, both antibodies present health complications. In the Alemtuzumab trial all but 10 % of patients experienced some sort of skin reaction. 21% of patients experienced a significant drop in white blood cell levels and 10% had cytomegalovirus develop as a result of treatment (Lundin et. al., 2002). Alemtuzumab is also known to cause Epstein-Barr virus, cytomegalovirus, immunosuppression and high infection rates, cytokine release syndrome, and neutropenia (Abbott, 2005; Kay et. al., 2002).

Rituximab showed much reduced side effects associated with a clinical trial. Two out of forty four patients showed high grade toxicity from treatment (Hainsworth et al., 2003). However, Rituximab therapy is associated with high infection rates of fungus and

bacteria, Herpes Simplex and pneumocystis (Abbott, 2005). There are significant therapy-associated risks for Complete Remission rates of only 19% and 4%, respectively.

Alemtuzumab and Rituximab have been added to other treatment regimens in hopes of enhancing their effects since they do not fair very well on their own (Byrd, 2006). They are employed as clean up treatments to get rid of any residual CLL cells that might be left behind after chemotherapy treatment. This approach seems to be more successful than MABs as monotherapies.

Clinical studies have tested Rituximab with Fludarabine and Cyclophosphamide, (alkylating agent) to create a combination treatment (FCR). One trial of 75 CLL patients resulted in a rate of 70% for complete responses that lasted for four years in 69% of the patients (Keating et. al., 2005). However, over half of patients in the trial developed a high level of neutropenia, one third acquired infections and 10% came down with fevers. Also, MABs killed off cells so rapidly that the accumulation of dead cells overloaded the kidneys.

Another trial of FCR showed 25% rates of CR in 177 patients undergoing relapse after treatment (Kay et al, 2002; Wierda et al., 2005). Twelve patients showed improvement at the bone marrow level. However, toxicity of treatment was high. Minor issues like fever, chills, hypotension, nausea and vomiting were common. Over half the patients developed high grade neutropenia. High grade thrombocytopenia and anemia and numerous cases of minor and major infections were observed in patients. Furthermore, while the use of MABs has shown up to 70% CR rate, it has not actually been shown to extend overall survival time of patients (Byrd, 2006).

Monoclonal antibodies are supplementary treatments that add a few percent to CR rates of chemotherapy regimens. However, MABs also add their own list of negative side effects to those of chemotherapy. Once again a CLL therapy that treats symptoms well does not achieve the greater goal of prolonging life.

Bone Marrow or Stem Cell Transplants

Chemotherapies are potent drugs that harm cells and tissues that demonstrate rapid division, including bone marrow (American, 2007). Marrow is attacked severely by chemotherapy to the point of blood cell depletion and major organ damage. Though chemotherapy can be more effective against CLL in higher doses, the side effects will not allow it.

Bone marrow or stem cell transplants (BMT, SCT) are used alongside chemotherapy as a way of salvaging the bone marrow (Rai et al., 2005). The process starts with administration of high doses of chemotherapy to CLL patients which cleans all stem cells out of the bone marrow. Then supplies of disease-free stem cells are transplanted, replenishing the blood supply.

Autologous SCT. With autologous transplantation, patients act as their own donor and have their own stem cells removed before chemotherapy (Canadian, 2006). These stem cells are cleaned of disease and preserved through freezing. After the high dosage chemotherapy, the purified stem cells are thawed and transplanted back into the patient.

Autologous transplants, or autografts, have not been shown to have superior survival rates to those of conventional therapies (American, 2007). A number of studies have concluded that autografts should not be recommended for treatment of CLL.

One such study treated 20 evaluable patients, 50% of which came out with progression-free survivals (Abbott, 2005; Khouri, Keating, Saliba & Champlin, 2002). There was an overall survival rate of 78%, and 80% of patients had complete molecular remissions. However, the majority of patients relapsed; the rate of second malignancy development was 8%, and high instances of serious infection were recorded. It was concluded that autologous stem cell transplantation did not provide an increase in overall survival rates.

In another study it took three separate autografts before 12 of 38 patients responded favorably to the treatment (Abbott, 2005). A synopsis of three autograft trials found low mortality rates related to the transplant (1-7%), but no plateau in progression-free survival. In other words, autografts were shown to be relatively tolerable but did not provide complete remissions.

Secondary malignancies and syndromes are also known to develop post-transplant. Secondary malignancies developed in 21% of patients in one study (from skin cancer to breast cancer) (Yee & O'Brien, 2006). Another study reported high chances of Myelodysplastic Syndrome development which can turn into Acute Myeloid Leukemia (Hamblin, 2005). All these risk factors show that autotransplantation is not a preferable treatment for CLL (Yee & O'Brien, 2006).

Allogenic SCT. The second type of SCT is allogenic transplants or allografts when stem cells are gleaned from a donor with a matching tissue type (preferably a sibling) (Rai et al., 2005).

Allogenic SCT is considered to be the only therapy that can actually cure CLL (Abbott, 2005). In contrast to Autologous SCT, allograft SCT does have a progression-

free survival plateau with CR rates as high as 87% and is therefore considered to be a CLL cure (Yee & O'Brien, 2006).

On the other hand, studies have shown allografts have a higher treatment related morbidity rate of 40- 50% (Hamblin, 2005; Yee & O'Brien, 2006). This means approximately half of patients treated with autologous SCT will die from it. One oncologist at Sunnybrook Hospital is quoted as saying that every patient over fifty years of age he has treated with an autograft from an unrelated donor has died within one month (D. Spaner, personal communication, 2007).

The main fatal side effect is Graft Versus Host Disease (GVHD), commonly known as transplant rejection (American, 2007). GVHD occurs when stem cells from a donor create an immune response that attacks self skin, liver, intestinal tissues, mouth, and other organs. This can result in loss of strength and energy, dry mouth, rashes and infections, soreness in muscles and in the most severe cases, death. Other side effects of allografts include low blood levels, vomiting, hair loss, cataracts, bone death (necrosis) and damage to the ovaries, thyroid gland and metabolism.

A clinical trial treated 28 CLL patients with cyclophosphamide and allogenic SCT (Khouri et al., 2002). For patients who had responded to previous chemotherapy, a 78% OS rate was seen and progression-free survival rate of the same percent was present for 5 years. However, in all patients there was a 49% chance of acute GVHD and an 11% morbidity rate at day 100 of treatment.

Stem cell transplants address the source of CLL by allowing for a complete wipe-out of stem cells from the bone marrow when combined with high dosage chemotherapy. The only problem is that neither form of SCT is ideal. Allogenic SCT

improves CR rates significantly but is associated with very high risks of transplant rejection and morbidity. At the other extreme, autologous SCT has much lower rates of fatality but considerably lower response rates. So it can be concluded that stem cell transplants are not a cure for CLL but rather treatments with weighty risks on one hand and low success on the other.

Potential Therapies

To date, no complete cure has been found for Chronic Lymphocytic Leukemia. Most treatments focus solely on symptoms and do not lengthen life. Other CLL therapies aim for complete remission but in the process can have intolerable side effects. CLL treatments have mutually exclusive results, either prolonging life with increased risk of unbearable side effects or treating symptoms while cutting life short.

CLL research seems to have a focus on chemotherapy, chemotherapy enhancers and chemotherapy combinations. So far, none of these have provided a desirable outcome. Study should therefore, branch out to new areas that show promise for potential treatment and cure of CLL.

Nanotechnology

A promising field of research for CLL therapy is nanotechnology (the employment and manipulation of substances that are a nanometer small) (National Cancer Institute, 2011). The hope is that a targeted method of attacking cancer cells can be created. If a nanodevice could be made to destroy cancerous cells in an exclusive, specialized way, a side-effect-free cure could be found. There are a number of different nanodevices currently under investigation.

Nanocarriers. Researchers have shown potential for nanocarriers to transport compounds like chemotherapy drugs exclusively to malignant cells, letting non-cancerous cells thrive and avoiding health risks like immunosuppresion normally associated with drug therapies (National, 2011). Nanocarriers allow for site-specific delivery of drugs so smaller amounts can be used and side effects lessened (Jain, 2010). Nanocarriers also prevent drugs from dissolving before reaching target areas and aid in delivery across membranes and barriers. Nanocarriers allow for tighter control of drug distribution and timing and ensure the drugs actually reach their target in their most potent form.

Passive nanocarriers are ideal for moving into tissues and building up specifically in leaky areas that are associated with cancerous growths (National, 2011). In the case of active nanoparticles, the cell surface make-up could cause the uptake of nanoparticles and attached drug into the cancer cells. The hope for both these types of devices is to allow for higher specificity and sensitivity of chemotherapy treatments by controlling drug targeting and delivery.

Nanoshells. There is also potential for a more active role for nanodevices apart from synthesized drugs. Nanoshells are currently under investigation for their potential to work inside cancer cells and induce cell death by absorbing light and emitting lethal heat (National, 2011). Cancerous cells would have to take in the nanoshells. Then light beams of specific wavelengths would be employed and the nanoshells would generate heat. The heat would kill off only the host cancer cells, leaving healthy cells unaffected.

This nanotechnology has actually been tested as a CLL treatment in the form of microscopic nanoshell beads coated with gold (Mukherjee et al., 2007; National, 2007).

In one study, the blood of CLL patients was isolated and tested for apoptosis levels in the presence of gold nanoparticles. Out of the samples tested, 40% showed significant levels of apoptosis of CLL B cells when just the nanoparticles were added. Higher apoptosis rates were observed when the nanoparticles were conjugated with antibodies.

Nanotechnology is a relatively new field for CLL treatment but shows encouraging signs of developing into a controlled therapy that could target only CLL cells, eliminating harmful side effects seen with conventional therapies.

Gene Therapy

Another potential CLL treatment under investigation is gene therapy. There are a number of possible ways scientists could alter the genetic makeup of a CLL patient (National, 2007). One possibility is to exchange mutated genes for healthy ones. Another approach is to work with the immune system. A combination of genes to weaken cancer cells with genes to make healthy stem cells resilient would make chemotherapy and other treatment more effective and less damaging. Finally, there is the possibility of inserting genes into CLL cells to induce apoptosis or to hinder formation of new cells.

Some risks are currently associated with gene therapy (National, 2007). One is that viral vectors used to transport and transmit genes may infect all cells instead of just diseased cancer cells. An immune reaction to the gene vectors could also occur which would be particularly devastating for immunocompromised CLL patients. Another risk is elevated expression with genes producing so many new cells that the body becomes overloaded.

Nanotechnology could play a role in creating effective genetic therapies for CLL (Bolhassani, Safaiyan & Rafati, 2011; National, 2011). Highly-branched dendrimer nanocarriers have been used to specifically target tumor cells for genetic therapy in mice (Chisholm, 2009). Injected dendrimers complexed with DNA accumulated at tumors and the attached gene was expressed specifically in tumor cells. Nanotechnology could provide a way to control potential side effects, making gene therapy an effective, safe CLL treatment.

Dendritic Cells

Dendritic cells are protein-sized molecules with a large surface area that present antigens to elicit an immune response from T cells and B cells (National, 2011). Dendritic cells are part of the protective microenvironment that supports CLL cells. A vaccine to elicit anticancer immune response could be created from CLL-associated antigens attached to a population of dendritic cells.

A treatment of nine patients with autologous dendritic cells recorded a decrease in leukemic lymphocytes (Hus et al., 2005). Another clinical trial treated twelve patients with autologous dendritic cells and five showed a decrease in the number of leukemic cells (Hus et al., 2008).

Nanocarriers could also be injected to transmit therapeutic drugs or DNA plasmids to dendritic cells present in the microenvironments surrounding CLL cells. Instead of injecting a vaccine of dendritic cells, nanodevices could target dendritic cells already in contact with CLL cells (Bolhassani et al., 2011).

Phytotherapy

Currently under investigation are a number of natural substances known for their anti-cancer properties. Extracts from neem tree leaf (Azadirachta indica) have been shown to have apoptotic and preventative effects against skin cancer in animals (Arora, Koul & Bansal, 2010). The extract is currently being employed in a CLL Phase I Trial by the Roswell Park Institute to test its effectiveness and toxicity as a CLL treatment (U.S. National Institute of Health, 2010).

A green tea extract has also been tested as a potential CLL treatment. Epigallocatechin-3-gallate (EGCG) is a protein inhibitor and was used in vitro with CLL B cells to determine the effects on cancerous cells (Lee et al., 2004). In 80% of the CLL samples tested, ECGG caused a notable increase in apoptosis. ECGG was shown to decrease the BCL-2 protein which is attributed with CLL cells' ability to avoid apoptosis. ECGG shows promise as an alternative CLL therapy or supplement to current treatments.

Finally, allicin, an extract from garlic, has been shown to have potential as therapy against B cell cancers (Arditti et al, 2005). This substance is produced when the enzyme allinase reacts with alliin. The allicin reaction was controlled and the allicin product was tested against CLL cells in vitro. It was used in combination with rituximab to target CD20+ CLL B cells. After treatment, B cell survival was reduced by 96%.

While most research as cancer therapy have been restricted to in vitro studies, natural extracts show potential as a source of effective CLL therapies with low toxicity. They may also one day be employed in combination with current therapies and enable lower dosage and decreased toxicity and negative side effects.

Summary

Conventional therapies for CLL are mostly temporary symptomatic treatments that combat the effects of the cancer and not the malignancy itself. As a result they are not proven to prolong life and in most cases, they add negative side effects to the disease load of CLL patients.

Chemotherapy

Chemotherapy drugs are the most widely used CLL treatment even though they can be highly damaging and only provide temporary relief of symptoms. Alkylator agents have low complete response rates with partial symptomatic improvement. They result in higher chances of secondary malignancies. Purine analogs, another group of chemotherapy drugs, yield higher rates of symptomatic relief. However, these therapies can result in severe immunosuppression and a CLL mutation into Richter's Syndrome.

Biologic/ Immunotherapy

Biologic monotherapies of monoclonal antibodies do not have as many side effects as chemotherapy but are also much less effective. When used in combination with chemotherapy, MABs increase effectiveness but also add more side effects to the previously mentioned risks of chemotherapy.

Bone Marrow or Stem Cell Transplant

There are two categories of stem cell transplants, both with their downsides. Though SCT is the only CLL therapy to address the root-cause of the disease, it is still not preferable. Allogenic stem cell transplants improve CR rates significantly but they have high risks of transplant rejection and transplant-related death. Autologous SCT have much lower morbidity rates but their response rates are not impressive.

Potential Therapy

Nanotechnology shows promise for controlled therapy that could exclusively target CLL cells and deliver drugs or heat to kill them without negative side effects.

If controlled by nanotechnology, gene therapy could manipulate DNA to weaken cancer cells, strengthen healthy cells and boost the immune system. Gene therapy has potential to be an effective and safe treatment of CLL.

Discoveries of natural cancer-fighting substances are being made in the field of phytotherapy. Natural extracts could treat CLL with low associated toxicity and risk.

Conclusion

In order to find a safe, complete cure for CLL, research needs to move away from a conventional, chemotherapy-centered focus. The symptoms and pathology of CLL are well researched and for the most part, understood. There are also numerous known factors that increase the risk of CLL. However, if a cure or preventative measures for CLL are to be discovered, the cause of the disease needs to be better understood.

Researchers should further investigate individual factors and the cumulative effects of genetic predisposition, precursor cells and antigen and chemical exposure. If the origin of CLL remains unknown, the search for a cure will continue to be nothing more than a hit-and-miss scenario guided by chance and not information.

Completely new areas with potential for permanent complete remission like nanotechnology, gene therapy and phytotherapy show promise as sources for effective, low toxicity CLL treatments. These and other safer, effective methods of treatment with potential for consistent results of permanent, complete remission should be further pursued.

References

Abbott, B. L. (2005). Advances in the diagnosis and treatment of Chronic Lymphocytic Leukemia. *Hematological Oncology, 23*(1), 34 – 40. doi:10.1002/hon.742

American Cancer Society, Inc. (2007). Overview: Leukemia - Chronic Lymphocytic (CLL). Retrieved from http://www.cancer.org/docroot/CRI/CRI_2_1x.asp?rnav=criov&dt=62

Arditti, F., Rabinkov, A., Miron, T., Reisner, Y., Berrebi, A., Wilchek, M., & Mirelman, D. (2005). Apoptotic killing of B-chronic Lymphocytic Leukemia tumor cells by Allicin generated in situ using a Rituximab-Alliinase conjugate. *Molecular Cancer Therapy, 4*(2), 325-31. Retrieved from http://mct.aacrjournals.org/content/4/2/325.long

Arora, N., Koul, A. & Bansal, M. P. (2010). Chemopreventive activity of Azadirachta indica on two-stage skin carcinogenesis in murine model. *Phytotherapy Research*. [Epub ahead of print]. doi:10.1002/ptr.3280

Bolhassani, A., Safaiyan, S. & Rafati, S. (2011). Improvement of different vaccine delivery systems for cancer therapy. *Molecular Cancer, 10*(3). Retrieved from http://www.molecular-cancer.com/content/10/1/3

Byrd, J. (2006). New drugs for Chronic Lymphocytic Leukemia. *Clinical Advances in Hematology & Oncology, 4*(3), 183-5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16728926

Chiorazzi, N., Rai, K. R. & Ferrarini, M. (2005). Mechanisms of disease: Chronic Lymphocytic Leukemia. *New England Journal of Medicine, 352*(8), 804-815. Retrieved from http://www.nejm.org/doi/full/10.1056/NEJMra041720

Chisholm, E., Vassaux, G., Martin-Duque, P., Chevre, R., Lambert, O., Pitard, B., Merron, A., . . . Baril, P. (2009). Cancer-specific transgene expression mediated by systemic injection of nanoparticles. *Cancer Research, 69*(6), 2655-2662. doi:10.1158/0008-5472.CAN-08-2657

Coleman, M., (Ed.). (2010). Understanding CLL/SLL. Lymphoma Research Foundation. Retrieved from http://www.cllinfogroup.org/atf/cf/%7B8ad9dbbf-45be-4641-944d-12b1d37dc7e0%7D/CLL_SLL10.PDF

Canadian Cancer Society (2006). Chronic Lymphocytic Leukemia Overview. *Canadian Cancer Encyclopaedia*. Retrieved from http://www.info.cancer.ca

Cataland, S., Lucas, M. & Byrd, J. (2005). Antibody therapy of acute and chronic Leukemias. *Hematology/American Society of Hematology Education Program,2*(4), 357-367. Retrieved from http://asheducationbook.hematologylibrary.org/cgi/content/abstract/2002/1/193

Dighiero, G., Maloum, K., Desablens, B., Cazin, B., Navarro, M., Leblay, R., Leporrier, M., . . . Travade, P. (1998). Chlorambucil in indolent Chronic Lymphocytic Leukemia. *New England Journal of Medicine,* 338. 1506-1514. Retrieved from http://asheducationbook.hematologylibrary.org/cgi/content/full/2005/1/278

Eichhorst, B., Busch, R., Stilgenbauer, S., Stauch, M., Bergmann, M., Ritgen, M., Kranzhöfer, N., . . . Hallek,M. (2009). First-line therapy with Fludarabine compared with Chlorambucil does not result in a major benefit for elderly patients with advanced Chronic Lymphocytic Leukemia. *Blood, 114*(16), 3382-91. doi: 10.1182/blood-2009-02-206185

Encarta® World English Dictionary [North American Edition] © (2007). Apoptosis, Deoxyrobonucleic Acid. *Microsoft Corporation.* Retrieved from http://encarta.msn.com/dictionary_561536658/apoptosis.html?partner=orp

Flynn, J. & Byrd, J. (2000). Campath-1H monoclonal antibody therapy. *Current Opinion in Oncology, 12*(6), 574-581. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11085457

Hamblin, T. J. (2001). Achieving optimal outcomes in Chronic Lymphocytic Leukaemia. *Drugs, 61*(5), 593-611. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11368285

Hamblin, T. J. (2005) Are we there yet?: Progress report. *Royal Bournemouth Hospital*. Retrieved from http://www.clltopics.org/PhysCor/TerryHamblinAWTY.htm

Hamilton, S. (2005). Chemotherapy drugs. *Cleveland Clinic Cancer Center.* Retrieved from http://www.chemocare.com/bio/

Hainsworth, J., Litchy, S., Barton, J., Houston, G., Hermann, R., Bradof, J., & Greco, F.A. (2003). Single-agent Rituximab as first-line and maintenance treatment for patients with Chronic Lymphocytic Leukemia or Small Lymphocytic Lymphoma: A phase II trial of the Minnie Pearl Cancer Research Network. *Journal of Clinical Oncology, 21*(9), 1746-1751. doi: 10.1200/JCO.2003.09.027

Hale, G., Bright, S., Chumbley, G., Hoang, T., Metcalf, D., Munro, A. J.& Waldmann, H. (1983). Removal of T cells from bone marrow for transplantation: A monoclonal antilymphocyte antibody that fixes human complement. *Blood, 62*(4), 873-882.

Hus, I., Roliński, J., Tabarkiewicz, J., Wojas, K., Bojarska-Junak, A., Greiner, J., Giannopoulos, K., . . . Schmitt M. (2005). Allogeneic dendritic cells pulsed with tumor lysates or apoptotic bodies as immunotherapy for patients with early-stage B-Cell Chronic Lymphocytic Leukemia. *Leukemia, 19*, 1621-1627. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15990861

Hus, I., Schmitt, M., Tabarkiewicz, J., Radej, S., Wojas, K., Bojarska-Junak, A., Schmitt, A., . . . Roliński J. (2008). Vaccination of B-CLL patients with autologous dendritic cells can change the frequency of leukemia antigen-specific CD8+ T cells as well as CD4+CD25+Foxp3+ regulatory T cells toward an antileukemia response. *Leukemia, 22*(5), 1007-1017. doi:10.1038/leu.2008.29

Jain, K. K. (2010). Advances in the field of nanooncology. *BMC Medicine 8* (83). doi:10.1186/1741-7015-8-83

Kay, N., Hamblin T., Jelinek, D., Dewald G., Byrd J., Farag S., Lucas, M., & Lin, T. (2002). Chronic Lymphocytic Leukemia. *Hematology, 2002*, 193-213.

Keating, M., Chiorazzi, N., Messmer, B., Damle, R., Allen, S., Rai, K., Ferrarini, M., & Kipps, T. (2003). Biology and treatment of chronic lymphocytic leukemia. *Hematology/American Society of Hematology Education Program.* 153-75. Retrieved from http://asheducationbook.hematologylibrary.org/cgi/content/full/2003/1/153

Keating, M., O'Brien, S., Albitar, M., Lerner, S., Plunkett, W., Giles, F., Andreeff, M., . . . Kantarjian, H. (2005). Early results of a chemoimmunotherapy regimen of Fludarabine, Cyclophosphamide, and Rituximab as initial therapy for Chronic Lymphocytic Leukemia. *Journal of Clinical Oncology*, *23*(18), 4079-4088.

Khouri, I. F., Keating, M. J., Saliba, R. M., & Champlin, R. E. (2002). Long-term follow-up of patients with CLL treated with allogeneic hematopoietic transplantation. *Cytotherapy, 4*(3), 217-221.

Kindt, T., Goldsby, R., & Osborne, B. (2007). Immunology. New York: W.H. Freeman and Company.

Lanasa, M. (2010). Novel insights into the biology of CLL. *Hematology/American Society of Hematology Education Program, (1),* 70-6. Retrieved from http://asheducationbook.hematologylibrary.org/cgi/content/full/2010/1/70

Lee, Y., Bone N., Strege A., Shanafelt, T., Jelinek, D. & Kay, N. (2004). VEGF receptor phosphorylation status and apoptosis is modulated by a green tea component, epigallocatechin-3-gallate (EGCG), in B-cell Chronic Lymphocytic Leukemia. *Blood 104*(3), 788-794. doi: 10.1182/blood-2003-08-2763

Lundin, J., Kimby, E., Björkholm, M., Broliden, P., Celsing, F., Hjalmar, V., . . . Österborg, A. (2002). Phase II trial of subcutaneous anti-cd52 monoclonal antibody Alemtuzumab (Campath-1H) as first-line treatment for patients with B-Cell Chronic Lymphocytic Leukemia (B-CLL). *Blood*, *100*(3), 768-773. doi: 10.1182/blood-2002-01-0159

Mayo Clinic. (2008). Chronic Lymphocytic Leukemia. http://www.mayoclinic.com/health/chronic-lymphocytic-leukemia/DS00565

Montserrat, E., & Moreno, C. (2008). Chronic Lymphocytic Leukaemia: A short overview. *Annals of Oncology, 19*(suppl 7), 320-325. doi:10.1093/annonc/mdn460

Mukherjee, P., Bhattacharya, R. L., Bone, N., Lee, Y. K., Patra, C. R., Wang S., Lu, L., . . . Mukhopadhyay, D. (2007). Potential therapeutic application of gold nanoparticles in B-Chronic Lymphocytic Leukemia (BCLL): Enhancing apoptosis. *Journal of Nanobiotechnology, 5(*4), doi:10.1186/1477-3155-5-4

National Cancer Institute. (2007). Chronic Lymphocytic Leukemia Treatment (PDQ). Retrieved from http://www.cancer.gov/cancertopics/pdq/treatment/CLL/Patient.

National Cancer Institute. (2011). Learn About Nanotechnology in Cancer. Retrieved from http://nano.cancer.gov/learn/index.asp

Nester, E., Anderson, D., Roberts, C. E., & Nester, M. (2007). Microbiology [A Human Perspective]. New York: McGraw-Hill.

Palma, M., Kokhaei, P., Lundin, J., Choudhury, A., Mellstedt, H., & Osterborg, A. (2006). The biology and treatment of Chronic Lymphocytic Leukemia. *Annals of Oncology, 17*(10), 144–154. doi:10.1093/annonc/mdl252

Rai, K., Keating, M., Larson, M., & Connor, R. (2005). Patient Information: Chronic Lymphocytic Leukemia. *UpToDate*. Retrieved from http://patients.uptodate.com/topic.asp?file=blod_dis/5404

Rai, K. R., Peterson, B. L., Appelbaum, F. R., Kolitz, J., Elias, L., Shepherd, L., Hines, J., . . . Schiffer, C.A. (2000). Fludarabine compared with Chlorambucil as primary therapy for Chronic Lymphocytic Leukemia. *New England Journal of Medicine 14*, 1750-1757.

Shaw, T., Quan, J., & Totoritis, M. C. (2003). B cell therapy for rheumatoid arthritis: the Rituximab (anti-CD20) experience. *Annals of the Rheumatic Diseases 62*(II):ii, 55–59. Retrieved from http://ncbi.nlm.nih.gov/pmc/articles/PMC1766758/pdf/v062p0ii55.pdf

U.S. National Institute of Health. (2010). Azadirachta Indica in Treating Patients with Chronic Lymphocytic Leukemia. Retrieved from http://clinicaltrials.gov/ct2/show/NCT01251250

U.S. News & World Report. (2008). About Chronic Lymphocytic Leukemia. *Mayo Clinic.* Retrieved from http://health.usnews.com/health-conditions/cancer/chronic-lymphocytic-leukemia#6.

Wierda, W. (2006). Current and Investigational Therapies for Patients with CLL.*Hematology*, 285-94. Retrieved from http://asheducationbook.hematologylibrary.org/cgi/content/full/2006/1/285

Wierda, W., O'Brien, S., Albitar, M., Faderl, S., Garcia-Manero, G., Thomas, D., Do, K.A.,… Keating, M. (2005) Combined Fludarabine, Cyclophosphamide, and Rituximab achieves a high complete remission rate as initial treatment for Chronic Lymphocytic Leukemia. *Journal of Clinical Oncology, 23*(18), 4070-4078. doi:10.1200/JCO.2005.12.516

Yee, K. W. L., & O'Brien, S. M. (2006). Chronic Lymphocytic Leukemia: Diagnosis and treatment. *Mayo Clinic Proceedings* 81(8), 1105-1129. doi:10.4065/81.8.1105