THERAPEUTIC OPTIONS FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Abstrak: Leukemia limfositik kronis (LLK) merupakan penyakit limfoproliferatif kronis yang ditandai oleh adanya akumulasi dan proliferasi sel B-matur, dengan fungsi kekebalan yang tidak kompeten, di dalam sumsum tulang, darah, kelenjar getah bening, limpa dan hati. Lebih dari 50% pasien tidak menunjukkan gejala pada saat terdiagnosis, dan biasanya tidak memerlukan pengobatan. Pada LKK yang lanjut dan progresif perlu diberikan pengobatan. Klorambusil dengan atau tanpa steroid telah merupakan obat pilihan selama bertahun-tahun pada pasien yang sebelumnya tidak pernah diobati sebagai LLK. Analog nukleosida purin, misalnya fludarabin, kladribin dan pentostatin juga telah diperkenalkan untuk pengobatan LLK. Namun, kemoterapi ini berisiko karena dapat menimbulkan konsekuensi problematis, seperti mielosupresi dan imunosupresi, yang dapat berakibat terjadinya infeksi oportunistik dan keganasan sekunder. Dewasa ini, penggunaan beberapa agen baru pada pengobatan LKK telah menunjukkan perkembangan yang menjanjikan, di antaranya adalah antibodi monoklonal. Selain itu transplantasi sel hemapoetik autologus dan alogenik semakin perlu dipertimbangkan pada pengobatan LKK, khususnya bagi mereka yang tidak dapat mentolerir terapi standar dengan dosis tinggi atau LLK yang berisiko tinggi. Penulisan ini difokuskan terutama pada strategi terapeutik LKK, termasuk mekanisme aksi obat serta respon terhadap pengobatan.
Kata kunci: leukemia limfositik kronik, kemoterapi, antibodi monoklonal, HSCT.

Abstract: Chronic lymphocytic leukemia (CLL) is a chronic B-lineage lymphoproliferative disorder, characterized by accumulation and proliferation of B-mature cells, which are immunologically incompetent, in bone marrow, blood, lymph nodes, spleen, and liver. More than 50% of patients are asymptomatic at the time of diagnosis, and they usually require no treatment. However, treatment is needed in the advanced and progressive stages of CLL. Chlorambucil with or without steroids has been the drug of choice for many years in previously untreated CLL patients. The purine nucleoside analogues, e.g. fludarabine, cladribine, and pentostatin, have also been introduced for the treatment of CLL. However, this chemotherapy is risky, because it can cause problematic consequences such as myelosupression and immunosupression, that can lead to opportunistic infections and secondary malignancies. Recently, several new agents appear promising in treating CLL, including new monoclonal antibodies. Moreover, using autologous and allogenic hematopoietic cell transplantations is increasingly being considered for the treatment of patients with CLL, especially for those who do not tolerate the high dose of standard therapies or suffer from high risk CLL. This paper focuses mainly on the therapeutic strategies in CLL, including the actions of the agents as well as the responses to the treatments.
Keywords: chronic lymphocytic leukemia, chemotherapy, monoclonal antibodies, HSCT.

As the most common leukemia among adults (25-30% of all leukemias), particularly among the elderly and middle age (60-70 years), the incidence in western hemisphere is around 3-50 cases/100,000 population/ year.[1] However, the incidence tends to incre-

ase in younger than 55 years patients, with nearly 30% of CLL cases.[2] In the USA, more than 150,000 new cases arose and there were 4,500 deaths because of CLL in 2007.[3]

CLL can be diagnosed when a lymphocyte count in peripheral blood is greater than 5 x 10^9/L, blood smear shows predominant small and mature-appearing lymphocytes, bone marrow aspiration exhibits >30% lymphocytes infiltration, and immunophenotyping is consistent with SmIg +/-, CD5+, CD19+, CD20+, CD23+, FMC7+/-, CD22+/-.[4]

The clinical staging system based on tumor load developed by Rai and Binet has been useful in estimating the prognostic factors in patients with CLL (table 1).[1]

In the past, CLL was assumed an incurable disease and so the people were expected to die mainly because of the disease rather than other complications related to CLL. Therefore, the treatment was not maximum and intended only as palliative. This traditional view was supported by randomized trials that showed no benefit in the initiation of alkylator-based therapy in early-stage CLL. However, as there have been evidence proven that most of CLL patients died due to complications related to CLL, optimal therapy has become a worthwhile goal in CLL treatment to achieve the best possible of complete remission and minimal residual disease eradication.[5]

CHEMOTHERAPY

Alkylating agent

Chlorambucil was the major therapy of CLL until early 1980s, and continues to be the main therapy for progressive CLL in older patients. Initial response rate of 60-90% and complete response of 20% patients were achieved in combination of chlorambucil and prednisone. However, the response rate depends on the doses, as the higher the dose the higher the response rates. Also in Binet stage A patients, chlorambucil does not extend the survival rate.[6]

Table 1. Summary of the Rai and Binet staging systems for chronic lymphocytic leukemia.[1]

RAI STAGING SYSTEM	
Stage 0 Stage 0 CLL is characterized by absolute lymphocytosis (blood: >15,000/mm³, bone marrow >40%) without adenopathy, hepatosplenomegaly, anaemia, or thrombocytopenia.	Low risk
Stage I Stage I CLL is characterized by absolute lymphocytosis with lymphadenopathy without hepatosplenomegaly, anaemia, or thrombocytopenia.	Intermediate risk
Stage II Stage II CLL is characterized by absolute lymphocytosis with either hepatomegaly or splenomegaly, with or without lymphadenopathy.	Intermediate risk
Stage III Stage III CLL is characterized by absolute lymphocytosis and anaemia (haemoglobin <11 g/dL) with or without lymphadenopathy, hepatomegaly, or splenomegaly.	High risk
Stage IV Stage IV CLL is characterized by absolute lymphocytosis and thrombocytopenia (<100,000/mm³) with or without lymphadenopathy, hepatomegaly, splenomegaly, or anaemia.	High risk
BINET CLASSIFICATION	
Clinical stage A[a] Clinical stage A CLL is characterized by no anaemia (Hb ≥10 g/dL) or thrombocytopenia (platelets ≥100,000/mm³) and <3 areas of lymphoid involvement.	
Clinical stage B[a] Clinical stage CLL is characterized by no anaemia (Hb ≥10 g/dL) or thrombocytopenia (platelets ≥100,000/mm³) with ≥3 areas of lymphoid involvement.	
Clinical stage C Clinical stage C CLL is characterized by anaemia (Hb <10 g/dL) and/or thrombocytopenia (platelets <100,000/mm²) regardless of the number of areas of lymphoid enlargement.	

[a]*Lymphoid areas include cervical, axillary, inguinal and spleen.*

Another alkylating agent used is cyclophosphamide, which has the same activity as chlorambucil, and commonly combined with doxorubicin, vincristine and prednisone. It is used if the tolerance to chlorambucil is poor. However, not many investigations have been done.[6] Alkylating agents work to add alkyl groups to negatively-charged groups. They are known to stop tumor growth through cross-linking guanine nucleobases in strands of DNA, which directly damages the DNA by making it unable to uncoil and separate. The cell, when attacked in this way, is unable to replicate. While it may not die, it also cannot grow.[7]

Monotherapy with purine analogous

The purine analogous usually used in CLL are fludarabine, pentostatine and cladribine, which administered through intravenous injection. However, the most common used is fludarabine. These three agents share similar chemical structures and mechanism of action such as induction of apoptosis. However, they exhibit also significant differences, especially in their interactions with enzymes involved in adenosine and deoxyadenosine metabolism activity. The cytotoxic mechanism of fludarabine and cladribine occurs via their phosphorylated derivatives, causing inhibition of ribonucleotide reductase and DNA polymerase, which results in decreased DNA synthesis.[8]

Fludarabine

Fludarabine (9-β-arabinofuranosyl-2-fluoroadenine monophosphate) in its nucleoside form (F-ara-AMP) is dephosphorylated to form F-ara-A, which is subsequently transported into cells and accumulates there as F-ara-ATP, that can inhibit ribonucleotide reductase, DNA polymerase, DNA primase and DNA ligase, all are required in DNA synthesis. So, fludarabine induces cell apoptosis of high proliferating cells in CLL patient.[9,10]

The administration of fludarabine is started in advanced stage CLL patients (Rai stages III/IV or Binet stage C, or Rai stages I/II or Binet stage A/B with symptoms of progressive disease). Compare to other conventional chemotherapies including cyclophosphamide, doxorubicin, vincristine, prednisone and chlorambucil, fludarabine induced more remission (7-40%) in the treatment of CLL patients in three phase III studies.[11] Nevertheless, in a meta-analysis study done by Zhu et all, the survival rate after 5-6 years follow up showed no significant difference between fludarabine and alkylator based regimens at conventional dose.[12]

This chemotherapy is risky, as it can cause problematic consequences, such as myelosuppression and immunosupression, that can lead to opportunistic infections and secondary malignancies.[13]

Therefore, the therapy based on immune concept has been developed since 20 year ago, when monoclonal antibodies in B-cell malignancies were first examined after the B-cell surface antigens had been discovered. A monoclonal antibody is supposed can eradicate tumor cells with a limited harm to normal tissues.[13]

MONOCLONAL ANTIBODIES

So far, there have been several monoclonal antibodies used and being investigated to against surface proteins of CLL cells, as shown in table 2.[14] CLL cells display CD markers 19, 20, 5, 23, 24, 26, 40, 79b, and sometimes 38.

Table 2. Monoclonal antibodies for chronic lymphocytic leukemia.[14]

Antibody	Antigen	Conjugate
CAMPATH-1H	CD52	None
Rituximab (Rituxan)	CD20	None
Epratuzumab	CD22	None
Hu-1D10 (Apolizumab)	HLA-DR	None
IDEC-152	CD23	None
IDEC-114	CD80	None
Bevacizumab	VEG-F	None
Denileukin diftitox (Ontak)	CD25	IL-2/diphtheria
BL-22	CD22	Pseudomonas

Rituximab

First found in 1997, rituximab as hu-

manized monoclonal antibodies (MAbs) targeting CD20 antigen on B-cells, was administered to patients with CD20-positive B-cell non-Hodgkin's lymphoma (NHL), either relapsed or refractory and low-grade or follicular.[15] In a little while, it was then tried to the CLL patients. Even so, in 11 investigations, rituximab showed lower response rate of CLL/SLL patients (10-15%) than of relapsed or refractory follicular NHL (46-58%). However, this could be caused by the low density of CD20 on these cells due to previous treatment. As Hainsworth et al observed higher significant response rate of CLL/SLL patients who have not been treated previously. Moreover, the period of the disease to become progressive after rituximab treatment was longer in patients with NHL compared to CLL/SLL.[15]

In a study by Thomas et al, single agent rituximab was given to 21 patients with early stage but high risk CLL (based on the elevation of β2-microglobulin), and it was found that overall response rates (ORR) were 90%, with 19% complete response (CR).[14]

Several efforts have been done to increase the effectiveness of rituximab, such as by rising the dose, managing the administration schedule more intensive or giving together with nucleoside analogues.[14] However, O'Brien et al revealed that the response rate did not correlate with the dose, after they tried to increase the dose from 375 mg/m^2 to 2,250 mg/m^2 in a phase I trial and found that the overall response was only 36% and ranged from 22% to 74%. Therefore, the combination of rituximab and chemotherapy has been developed.[6]

One of the studies using combination rituximab and chemotherapy was carried out by the cancer and leukemia group B (CALGB), who administered concurrent and sequential rituximab and fludarabine to 51 and 53 patients, respectively, in a randomized phase II trial. Concurrent regimen showed 90% ORR including 47% CR, while sequential arm exhibited 77% and 28%, respectively. So, this complete responses were by far higher than that of fludarabine alone.[13,14]

Alemtuzumab

Alemtuzumab is also a humanized monoclonal antibody that works directed to CD52 antigen. CD52 express on all lymphocytes at various differentiation stages, with the highest levels on T-prolymphocytic leukemia (PLL) cells, after that B-CLL and the smallest amount appear on normal B cells. Also, could be found on monocytes, macrophages, eosinophils, and reproductive tract. The later as non-lymphoid site, is only displayed at males. On the other hand, this antigen does not appear on other hematopoietic cells including stem cells, erythrocytes and platelets.[13]

The alemtuzumab acts at several mechanisms, consist of antibody dependant cellular cytotoxicity, complement mediated cytotoxicity, and apoptosis induction. Nowadays, there are two brands of alemtuzumab used widespread, first an IgM antibody (known as CAMPATH-1M) and second CAMPATH-1G, a murine derivative, which exhibits clinical activity in refractory CLL particularly to patients who do not response well to CAMPATH-1M.[13]

The dose administered to patients is firstly 30 mg at day I, then if the patient tolerates well, it is increased to 10 mg at day II, and continues to 30 mg, three times per week started at day III, as long as the infusion related reactions are acceptable, until the period of maximum 12 weeks.[6,13] However, several researches reported that patients could attain additional advantage if the duration of alemtuzumab treatment becomes longer.[13]

In a pivotal test by Keating et al, 33% ORR and 2% CR were accomplished after the administration of 30 mg alemtuzumab intravenously was managed to 93 fludarabine-refractory CLL patients. The median time to progression extended to 10 months, and overall survival was 32 months.[5] Different studies showed overall response rate ranged between 31% and 60%, and complete response rate between 0 and 31%. In common, these studies confirmed that alemtuzumab, as an antitumor, acts better in bone marrow and blood rather in lymph nodes.[6]

One should be alert in alemtuzumab management is the incidence of infections, such as *Pneumocystis carinii* pneumonia, herpes simplex virus and cytomegalovirus, which is usually observed in patients receiving alemtuzumab. Therefore, to prevent patient from these opportunistic infections, prophylaxis treatment consisting of antibiotics, such as trimethoprim-sulfamethoxazole, and antiviral, for example acyclovir, famciclovir or valaciclovir, are obligatory.[6,13] This prophylactic regimen is given until 2-4 months after alemtuzumab ceased or when the CD4+ lymphocyte counts has reached a minimum of 250 x 10^9/µl.[6]

HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

To date, stem cell transplantation has been improved as the treatment to those who do not tolerate with high dose of standard therapies or have high-risk CLL, in order to lengthen the life expectancy or even cure the disease. However, there have been no studies that compare exactly stem cell transplantation with conventional therapy.[16]

Two types of hematopoietic stem cell transplantation are autologous, in which the stem cells derived from the patient's own stem cells, and allogenic, where the stem cells obtained from a donor. Autologous is better than allogenic HSCT in terms of graft-versus-host disease (GVHD). On the other hand, because of this lack of GVHD, autologous has greater possibilities of disease to relapse, as the autograft contaminates with malignant cells.[17] Other problems that can occur after autologous transplantation are late complications of CLL, for instance acute myelogeneous leukemia and myelodysplasia, and no evidence of plateau on overall and disease-free survival (DFS).[6,17]

Milligan et al performed a prospective study, which treated patients with autologous HSCT after the administration of fludarabine, and found that overall survival was 67%, early transplant mortality was only 1.5%, and complete response increased from 37% to 74%, also 63% patients achieved the complete response after transplantation. The five-year survival was 77.5% and disease-free survival rate was 51.5%. However, the detection of molecular remission by using Polymerase Chain Reaction showed molecular relapse is likely to occur in many patients.[16,17]

In allogenic hematopoietic stem cell transplantation, as the stem cells from donor must be similar to the recipient's, HLA typing is used to identify the perfect donor. HLAs, the proteins on the surface of cells, facilitate the immune system to recognize whether a cell comes from outside or truly belongs to the body. In the case of CLL, the transplantation tends to succeed and therefore increases the survival, if the recipient HLA-A, HLA-B, HLA-C, HLA-DRB1 and HLA-DQB1 match to the donor's.[17]

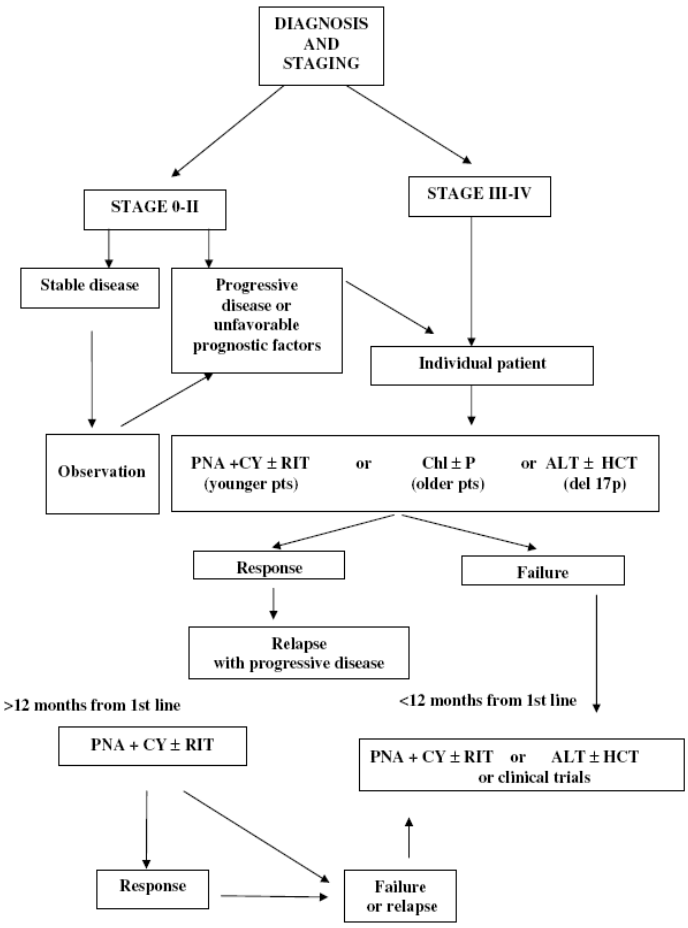

Figure 1. Proposed treatment algorithm for CLL patients.[6] (PNA= purine nucleoside analogues, CY=cyclophosphamide, RIT=rituximab, ALT=alemtuzumab, Chl=chlorambucil, P=prednisone, HCT=hematopoietic stem cell transplantation).

It has been proven that a sibling has a 25% chance of HLA-identical match, and they are called a related donor if they have class I and class II HLA-match. Another category termed as alternate stem-cell donors, are those who are unrelated donors, unrelated umbilical-cord blood donors and partially (haploidentical) matched family donors, but have HLA-match to the recipient. Usually the transplantation from matched-sibling donor produces better outcome than from unrelated donors, mainly caused by graft rejection and GVHD reasons.[17]

Unfortunately, there has been no randomized trial that evaluates what time is better to do transplantation, either at early or late phase of disease, although retrospective studies have shown that transplant in the early phase results in better outcome. Nevertheless, this indicates HSCT should be combined with the conventional therapy after first complete or partial response in selected patients.[17] A proposed treatment for CLL patients is summarized in Figure 1.[6]

CONCLUSION

As most of CLL patients died due to complications related to CLL, optimal therapy has become a valuable goal in CLL treatment to achieve the best possible of complete remission and minimal residual disease eradication. Chlorambucil has been the major therapy of CLL and continues to be the main therapy for progressive CLL in older patients, in practice. The purine nucleoside analogs, including fludarabine, pentostatine and cladribine, also have been administered in advanced stage CLL patients (Rai stages III/IV or Binet stage C, or Rai stages I/II or Binet stage A/B with symptoms of progressive disease). However, this chemotherapy can cause problematic consequences, such as myelosuppression and immunosupression, which results in opportunistic infections and secondary malignancies.

Therefore, the therapy based on immune concept has been developed, and this monoclonal antibody is supposed can eradicate tumor cells with a limited harm to normal tissues. Rituximab as humanized monoclonal antibodies (MAbs) targeting CD20 antigen on B-cells, shows higher complete response rate when combining with chemotherapy. Alemtuzumab is also a humanized monoclonal antibody that works directed to CD52 antigen, and acts better in bone marrow and blood rather in lymph nodes. However, the incidence of opportunistic infections is usually observed in patients receiving alemtuzumab. Therefore, prophylaxis treatment consisting of antibiotic and antiviral, are obligatory.

Recently, autologous and allogenic hematopoietic stem cell transplantations are gradually considered as the treatment to the patients who do not tolerate or fail with standard therapies or have high-risk CLL.

In brief, attempts to further intensify therapy are ongoing, particularly in immune-based strategies including new monoclonal antibodies and cellular therapies, in order to offer the best therapeutic options for the benefit of patients.

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