Proceedings of the Meeting on
CHRONIC LYMPHOCYTIC LEUKEMIA:
IS IT A CURABLE DISEASE?
October 10-11, 2002
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CHRONIC LYMPHOCYTIC LEUKEMIA: IS IT A CURABLE DISEASE?

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CLL: Is it a curable disease?

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The capacity of neoplastic cells to respond to selected microenvironmental signals confers a growth advantage and extended survival to chronic lymphoid malignancies of the B-cell type. Chronic lymphocytic leukemia (CLL), which is characterized by the expansion of monoclonal CD5+ B lymphocytes, is a prototype example because malignant cells accumulate in secondary lymphoid organs, bone marrow (BM) and peripheral blood (PB) not only because of genetic lesions but also thanks to their interactions with non-tumoral bystander cells. Accessory cells and T-lymphocytes appear to play a critical role in the CLL cell/microenvironment cross-talk. A direct physical contact between BM stromal cells and leukemic cells extends the CLL cell survival. Follicular dendritic cells are closely associated with CLL cells in the early phase of BM involvement. Moreover the PB of CLL patients has been shown to contain cells that in vitro can differentiate into adherent nurse-like cells, endowed with the capacity of protecting the attached leukemic B-cells from spontaneous apoptosis. The absolute number of T-cells is increased in CLL patients and T-cell subsets are redistributed with CD4+ T-cells predominating in involved BM and lymph nodes. The T-cell receptor (TCR) repertoire frequently shows an oligoclonal pattern and several T-cell cytokines (including interleukin-4, interferon-α and interferon-γ) are able to inhibit CLL cell apoptosis. Many reports suggest that CLL cells and T-cells may be involved in a reciprocal dialogue via CD40/CD40ligand (CD40L) interactions since the stimulation of CD40 rescues CLL cells from apoptosis and induces their proliferation.

CLL is currently interpreted as an accumulative disorder. The relentless increase of malignant cells is due to defective apoptosis that causes extended cell survival. More than 99% of circulating CLL lymphocytes are in the G0/early G1 phase of the cell cycle, are unresponsive to the exogenous stimuli that favor the cell cycle progression of normal B-cells and their B-cell-receptor (BCR) organization tends to prevent BCR-mediated activation. Notwithstanding their resting kinetic state, PB CLL cells express membrane markers of cellular activation, have the mRNA for a wide variety of cytokines and actually secrete several of them as activated B-cells do. The accumulation compartment is nourished by an upstream proliferation compartment represented by focal aggregates of proliferating cells that form the so-called pseudo-follicles in lymph nodes and are scattered in the BM. CD4+ cells tend to concentrate around and within the proliferating pseudo-follicles and are activated, as witnessed by the frequent expression of CD40L.

These data lead to the questions of what are the relationships between proliferation and defective apoptosis and through which molecular pathways does the microenvironment exert its influence on the malignant clone? A link has been established between the CD40/CD40L pathway and the expression and modulation of survivin, a prominent member of the family of inhibitor of apoptosis proteins (IAP), that integrates apoptosis and proliferation. The expression of survivin is absent in resting PB CLL cells but can be induced in vitro by CD40 stimulation. Survivin+ cells acquire an extended survival and an increased rate of proliferation. In vivo survivin+ cells are localized in lymph node pseudo-follicles and in rare BM clusters of proliferating CD5+ B-cells that are interspersed with T-cells, essentially of the CD4+ type and fre-
quenty CD40L$^+$ indicating the in vivo availability of this signal to leukemic cells.\textsuperscript{11}

The next obvious question becomes: how do malignant B-cells and T-cells come into close proximity? CLL cells purified from involved lymph nodes and BM, but not from PB, constitutively express mRNA for the T-cell attracting chemokines CCL17 and CCL22.\textsuperscript{13} CD40-crosslinking of PB CLL cells induces the expression of both chemokines at RNA level. CCL22 is also released and is capable of attracting CD4$^+$ CD40L$^+$ T-cells. These findings indicate that the stimulation of malignant cells via a physiologic signal present in the tumor microenvironment endows CLL cells with the chemotaxic capacity for activated CD4$^+$ T-cells, which in turn can deliver survival signals to tumor cells.\textsuperscript{13}

Further studies aimed to identify which molecular interactions link malignant B-cells, T- and accessory cells and provide the signals important for the extended survival and proliferation of CLL cells have led us to focus upon CD100 (recently renamed Semaphorin D), a transmembrane protein belonging to the fourth group of the semaphorin family, and its receptors, CD72 (low affinity receptor) and Plexin-B1 (high affinity receptor). To this end we first studied the expression and functional role of the semaphorin CD100 in CLL PB and BM and normal CD5$^+$ B-cells obtained from tonsil samples. We then asked whether CLL cells might be exposed to the high affinity receptor Plexin-B1 in the tissues where they usually accumulate during disease progression (i.e. lymph nodes and BM). Finally, to address the question of whether Plexin-B1 could be a receptor for CD100 within the immune system, we co-cultured CLL cells (or normal CD5$^+$ B-cells) together with Plexin-B1 transfectant cell lines. The data indicate that: i) CD5$^+$ normal and leukemic B-cells uniformly express CD100; ii) Plexin-B1 is available to CD100$^+$ lymphocytes in different specific microenvironments, as it is expressed in BM stromal cells, follicular dendritic cells and activated T lymphocytes; iii) CD100/Plexin-B1 interactions deliver survival and proliferation signals to both normal and leukemic CD5$^+$ B-cells; iv) the expression of CD100 is upregulated by CD40 stimulation and the co-stimulation of CD100 and CD40 provides additive signals. Taken together these findings suggest that CLL cells utilize the supportive interactions which allow the successful social life of normal B-cells and retain the capacity to respond to proliferative and anti-apoptotic microenvironmental signals provided by bystander cells through cellular contacts.

Such a scenario provides the conceptual framework to develop new treatment modalities aimed at interrupting the interactions between malignant B-cells and the microenvironment.

References


In Western countries leukemia affects ~1-2% of the population. Of the many subtypes, B-cell chronic lymphocytic leukemia (CLL) is the most common, constituting about 30% of all cases. Epidemiological studies strongly suggest that CLL and other lymphoproliferative disorders have an inherited component. There are over 40 reports in the literature describing the clustering of CLL, sometimes in association with other leukemias or lymphomas. The transmission of CLL in these families is most parsimonious with an autosomal dominant mode of inheritance with incomplete penetrance. Five case-control studies and one cohort study have found that the risk of leukemia or other lymphoproliferative disorders in relatives of lymphocytic leukemia patients is increased 3-fold.

Early major contributions were made by Videbæk in Denmark and Gunz et al. in Australia. Videbæk wrote a very comprehensive monograph raising the issue of familial leukemia and noted that of all leukemias, CLL is the one in which a hereditary component is most apparent. The subject was reviewed by our group and details of the research methodology necessary to approach this problem were described. Four years ago we undertook a genetic study of familial CLL based initially on the distribution of family history questionnaires; 625 CLL patients have so far replied, and of these 318 (51%) have completed the questionnaire.

A positive family of CLL or other leukemia or lymphoma has been documented in 30%: 16% CLL, 5.6% other leukemia, 5% non-Hodgkin’s lymphoma and 2.2% Hodgkin’s disease. From this group we have information on 225 families with CLL: 11 have 4 or 5 affected members, 27 have 3 (Figure 1, family pedigrees), 80 have 2 affected siblings, 81 have a parent and child affected and 5 a grandparent and grandchild affected. We have thus far collected 72 samples useful for a genetic linkage study, i.e. DNA from two or more affected members of a family. This methodology is similar to that used to discover the breast cancer susceptibility genes and now includes analysis of germline DNA (mouthwash samples) in addition to leukemic cell DNA. The chances of CLL occurring in 3 or 4 members of a family are extremely small and would be expected by chance to occur every 1,000 years. Twenty-four of 50 familial CLL cases published had 3 or more affected members (Figure 1). Therefore the existence of such families strongly supports the concept that there is a genetic/hereditary component with evidence of vertical transmission in a proportion of CLL cases. This is consistent with the expression of an autosomal dominant gene.

Our international collaboration has continued in Copenhagen with Viggo Jønsson who has endeavoured to follow the family pedigrees reported by Videbæk in 1947. Pedigree 14 was described with 2 cases of CLL and one of another leukemia in 2 generations. The painstaking work of Jønsson has now discovered 2 more cases in the 3rd and 4th generations plus an indolent T-cell lymphoma. Clearly, the published reports, case-control studies and cohort studies show a 30-fold increase in risk in relatives of patients, which was confirmed in our own survey. The main task now is to pursue the collection of families more vigorously. Various results regarding candidate genes have already emerged from our study.

We and others have confirmed the phenomenon of anticipation that was described in CLL and acute myeloid leukemia by Horwitz et al. but had already been suspected by Videbæk. In a recent study we investigated whether nucleotide repeat expansion is a feature of CLL by means of the repeat expansion detection (RED) technique. We found no evidence of pathologic CAG expansions and therefore concluded that this mechanism, common in other hereditary disorders, is unlikely to be involved in the phenomenon of anticipation.
in familial CLL.

The possible role of ATM mutations in CLL has been a central theme of our research efforts. To examine the proposition that ATM mutations are involved in CLL we have analyzed a series of 28 families segregating CLL to test for genetic linkage using a series of microsatellite markers. There was no evidence for linkage, with allele sharing probabilities being identical to those randomly expected. To assess the role of germline ATM mutations in familial CLL directly, we recently screened 61 affected individuals from 29 CLL families for constitutional mutations. Two individuals had truncating ATM mutations and six missense ATM mutations were identified. There was evidence of co-segregation of these mutations in the families implying that they only confer small genotypic risks.

In addition to ATM we did not demonstrate genetic linkage in familial CLL for major histocompatibility complex (MHC) loci (chromosome 6p21.3) and the pseudoautosomal regions (chromosomes Y and X).

Very recently, in collaboration with the Leeds group, we have detected a subclinical CLL-phenotype in 13.5% (8/59) healthy first degree relatives of patients from 21 families with two or more cases of CLL using a sensitive flow cytometry method that detects the higher CD5 and lower CD20/CD79b expression of CLL lymphocytes. This finding represents a highly significant increase in risk (compared with 3.5% expected in normal individuals) and provides a new and valuable surrogate marker of carrier status.

References

B-cell chronic lymphocytic leukemia (CLL) accounts for approximately 30% of reported leukemia cases in Western countries. It has an incidence of 2.3-3.3 cases per 100,000 people. Even though the disease more frequently affects elderly people (median age 60 years), a significant fraction of cases (10-15%) is diagnosed in subjects younger than 50 years old. A growing body of evidence has been accumulated over the last decade demonstrating the variability of the clinical course of this disease. This variability may reflect underlying differences in phenotypic and molecular cytogenetic features. The introduction of B-cell mitogens, along with the development of sensitive molecular cytogenetic techniques, has helped us to extend our knowledge on the cytogenetic profile of CLL significantly.

The salient information deriving from cytogenetic and molecular cytogenetic studies are summarized here.

Cytogenetic findings

A number of studies performed over the last 20 years showed that approximately 20-50% of CLL carry a clonal chromosome defect. The variability in the incidence of cytogenetic aberrations is accounted for by the heterogeneity of the population of patients, by different culture conditions and by the timing of cytogenetic analysis, the probability of detecting abnormal dividing cells being higher at disease progression. A large cooperative study reporting a >50% incidence of chromosome anomalies included over 400 patients, only 29% of whom were studied at diagnosis. It is worth noting that CLL is a cytogenetically stable disease with less than 20% of the patients acquiring additional defects during the history of the disease.

Approximately 50% of abnormal cases carry a single chromosomal defect, 25% show 1 or 2 defects and 20-25% carry a complex karyotype (3 or more aberrations in the same clone). In a multicenter study, 40-50% of the patients had a normal karyotype (NN karyotype), 40-50% had 1-99% abnormal metaphases (AN karyotype) and 10% showed only abnormal metaphases (AA karyotype). The number of clonal abnormalities (complex karyotype) and the AA karyotype status (AA vs AN vs NN) represent two important prognostic factors in univariate analysis, the latter maintaining significance in multivariate analysis. These findings support the assertion that genetic stability is an important prognostic factor in human neoplasias. Clearly, the in vitro mitotic index of the cytogenetically abnormal clone can reflect its in vivo growth potential, accounting for the association of the AA karyotype with short survival.

Specific chromosome aberrations are associated with peculiar clinicobiological features (Table 1).

When comparing survival probability according to specific chromosome anomalies, those patients with a normal karyotype and with 13q- do better than those patients with +12 and 11q-, the worse outcome being associated with 14q anomalies. Most of the patients with 14q anomalies have CLL/PL sharing clinicobiological features with leukemic mantle cell lymphoma.

The difference in survival probability is maintained when analyzing those patients with a single clonal aberration, definitely showing that 13q, occurring as a single aberration, and normal karyotype have a favorable prognostic significance and that +12 and 11q- represent unfavorable cytogenetic markers. The acquisition of +12 is an early cytogenetic event in the history of CLL, although it probably is not the primary anomaly. Indeed, the presence of +12 and of 13q deletion in two distinct populations of neoplastic lymphocytes...
belonging to the same patient suggest that these cytogenetic aberrations may be superimposed on an, as yet, unidentified submicroscopic primary change. The findings that +12 cells preferentially home to the lymph node and bone marrow, that the cells are not reduced or eliminated by chemotherapy11 and that their population expands as disease progresses, clearly support the hypothesis that this anomaly plays an important role in the natural history of the disease.

Some authors have described unequal distribution of clonal chromosome anomalies at different sites12 [bone marrow, peripheral blood and lymph nodes]. Lymphocytes carrying +12 were found to accumulate preferentially in the lymph node. Unequal distribution of cytogenetically abnormal cells at different sites involved by disease has recently been observed also in myeloid neoplasias,13,14 possibly reflecting selective retention and/or destruction of leukemia cells due to as yet unclear mechanisms. In some cases different properties of adhesion to bone marrow or lymph node stromal cells have been postulated to play a role in this process.15

There are some recurrent chromosomal defects occurring in 1% or less of CLL. The significance of these is being gradually elucidated.16, 17 Interestingly, some of these chromosomal changes are usually associated with therapy-demanding disease and a relatively aggressive clinical course (Table 2).

Molecular cytogenetic data

Because the mitotic index in CLL is low, conventional cytogenetic analysis can only detect aberrations in 50% of the cases, when the analysis is performed at diagnosis and during follow-up. In addition, inadequate banding resolution may preclude unequivocal recognition of subtle rearrangements which can be correctly identified by fluorescence in situ hybridization (FISH). Comparative genomic hybridization (CGH) studies are of value in the identification of chromosomal gains and losses in the magnitude order of at least 10-20 Mb.

Using a panel of 4 probes which detect the 13q14 deletion distal to the Rb gene, the 11q22.3-23.1 deletion involving the ATM gene, the 17p13.3 deletion involving p53 and total/partial trisomy 12 centred around the 12q13 segment, 70-75% of CLL can be shown to carry a cytogenetic lesion. This figure may rise to 82% if additional probes are employed which recognize 6q21 deletion, 14q32 translocations, 3q and 8q partial trisomy.18

Using a hierarchical classification giving primary importance to 17p-, followed by 11q-, +12 and +7, Dohner et al.18 found that the incidence of these cytogenetic groups was 7%, 17%, 14%, and 36%, respectively, the remaining cases having other aberrations (8%) or a normal karyotype (18%). Clinical outcome in these cytogenetic groups is significantly different, the shortest survival having been observed in those patients with 17p- (32 months) and 11q- (79 months). The remaining patients with a normal karyotype, +12 and 13q- were found to have a median survival in the range of 111-133 months. There are at least two possible explanations for the discrepant results in terms of prognostic significance of +12 by cytogenetic

Table 1. Incidence and significance of chromosome abnormalities in CLL.

<table>
<thead>
<tr>
<th>Aberration</th>
<th>Frequency*</th>
<th>Hematologic features</th>
</tr>
</thead>
<tbody>
<tr>
<td>13q14 deletion</td>
<td>10-50%</td>
<td>Favorable prognosis and typical morphology if present as the sole change</td>
</tr>
<tr>
<td>+12</td>
<td>15-25%</td>
<td>CLL mixed-cell type; therapy-demanding disease; relatively short survival if detected by cytogenetic analysis</td>
</tr>
<tr>
<td>11q21 deletion</td>
<td>5-15%</td>
<td>Typical CLL, karyotype instability; ATM gene involvement; massive lymphadenopathy, young age, short survival</td>
</tr>
<tr>
<td>17p deletion</td>
<td>1-5%</td>
<td>CLL/PL; advanced disease, very short survival; refractory to purine analogs</td>
</tr>
<tr>
<td>Deletions at 6q</td>
<td>3-6%</td>
<td>Atypical morphology; high WBC count</td>
</tr>
<tr>
<td>t(11;14)(q13;p22)</td>
<td>Rare</td>
<td>Only CLL/PL; overlapping features with leukemic mantle cell lymphoma; splenomegaly</td>
</tr>
<tr>
<td>t(14;19)(q32;q13)</td>
<td>Rare</td>
<td>Atypical CLL; aggressive disease</td>
</tr>
</tbody>
</table>

*Wide variation in percentages due to the sensitivity of the method, i.e. conventional chromosome analysis or molecular cytogenetic techniques, and the heterogeneity of the populations of patients.

Table 2. Other recurrent chromosome aberrations in CLL.

<table>
<thead>
<tr>
<th>Chromosome defect</th>
<th>Additional defects</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>6p24-25</td>
<td>11q-; +12; 6q-; 17p</td>
<td>aCLL; early stage, disease progression</td>
</tr>
<tr>
<td>4q21</td>
<td>6q-; +12; 17p</td>
<td>CLL/PL; therapy-demanding disease</td>
</tr>
<tr>
<td>12p</td>
<td>none</td>
<td>Typical CLL; early stage; stable disease</td>
</tr>
<tr>
<td>21p</td>
<td>13p</td>
<td>aCLL; disease progression</td>
</tr>
<tr>
<td>1p34</td>
<td>Variable</td>
<td>Typical CLL; early stage, indolent disease</td>
</tr>
<tr>
<td>4q35</td>
<td>Variable</td>
<td>aCLL; therapy-demanding disease, paraproteinemia</td>
</tr>
<tr>
<td>4p16</td>
<td>Variable</td>
<td>Typical CLL; disease evolution</td>
</tr>
<tr>
<td>9q-</td>
<td>+12</td>
<td>aCLL, indolent disease</td>
</tr>
<tr>
<td>+7</td>
<td>+12</td>
<td>Few cases</td>
</tr>
</tbody>
</table>

by 13q-,
analysis (highly significant) and by FISH (not significant). First, cytogenetic analysis can identify only those cases with +12 with a relatively high mitotic index, whereas FISH may also reveal those cases with +12 in interphase cells. Second, FISH can detect minor clones which escape detection by cytogenetic analysis due to the limited number of metaphases analyzed. The proliferative capacity of the neoplastic clone and its size in vivo represent two markers of disease activity.

The combination of morphologic, immunologic and cytogenetic studies can identify distinct disease profiles in CLL which may prove useful in a clinical setting. The salient cytogenetic and clinicobiological correlations are summarized in Table 1. A novel cytogenetic and clinicobiological association is represented by the 6q21 deletion, which was recently shown to cluster at a 3-cM region and to be associated with CLL mixed-cell type presenting with a relatively high white blood cell count and a therapy demanding disease. A growing body of evidence has been provided over the last years showing that the variability of the clinical behavior in CLL is mirrored by biological heterogeneity. Two novel important markers were shown to have strong prognostic importance, namely the mutational status of the Ig gene variable regions and the expression of CD38 antigen. Approximately 50% of CLL harbor hypermutated Ig gene configurations, reflecting an origin from a post-germinal center B-cell, whereas the remaining cases do not show such mutations because they derive from a pre-germinal center CD5+ B-cell or from a cell that has encountered the antigen in a T-cell independent reaction. There is a preferential, though not absolute, association between CD38 negativity and hypermutated CLL and between CD38 positivity and unmutated CLL. The former group of CLL has a better outcome than the latter group and, as expected, the unfavorable cytogenetic categories (17p-; 11q-) tend to cluster in the unmutated CD38+ category. In conclusion, cytogenetic analysis and FISH studies provide important information in the work-up of CLL in that they: a) identify novel rearrangements; b) are associated with distinct disease subsets and; c) allow for a refinement of risk assessment.

The combination of classical clinical parameters (staging systems), and immunologic, cytogenetic and genetic characteristics helps to divide CLL into different disease subsets requiring specific treatments.

References


Chronic lymphocytic leukemia (CLL) is a disease characterized by the accumulation of resting neoplastic B-lymphocytes in bone marrow, lymphoid tissues, and peripheral blood. In addition, CLL is associated with immune disturbances such as hypogammaglobulinemia and immune cytopenias. Survival of patients with CLL is highly variable, with some patients living as long as sex- and age-matched controls and others dying soon after diagnosis. In a proportion of cases, CLL evolves into more aggressive lymphoproliferative disorders. Although CLL is in most instances an incurable disease, important progress has been made in its management. The variability in the prognosis, as well as the different treatment options, make it necessary to treat patients with CLL according to their individual risk.1-3

Over the last 15 years a number of changes have taken place in the characteristics of CLL at diagnosis as well as in its evolution,4, 5 making it worthwhile to review the natural history of this form of leukemia and how this affects its management.

Incidence and demographic characteristics

CLL is the commonest form of leukemia in Western countries, accounting for 20-30% of all leukemias. In contrast, this form of leukemia is infrequent in Asian countries (< 5% of all leukemias). Interestingly, in Asian people who have emigrated to Western countries the incidence of CLL does not increase over generations. Familial cases of CLL are not rare. An interesting phenomenon in familial CLL is the fact that in affected members of the second generation the disease presents 10-15 years earlier than in the first members of the family.6

CLL is a disease of the elderly. Thus, it is extremely rare in individuals under the age of 40 (<5 new cases/100,000/year), whereas in persons above 70 this form of leukemia is very common (30 new cases/100,000/year) (Surveillance and Epidemiology End Results, NIH). In all series, males predominate over females (1.5-2:1). Interestingly, whereas in patients with early stage disease (i.e., Binet A) there is no gender predominance, in advanced stages (i.e., Binet B and C) males outnumber females.

Over the last decade, the median age of patients at diagnosis has increased from 60-65 to 70 years. This is due to the predominance of the disease among the elderly and to the prolongation of life-expectancy in the general population. As a result, about one third of the patients are older than 70 at diagnosis. From the treatment standpoint, the latter is a highly neglected population, a fact that should be corrected. At the same time, the proportion of CLL cases detected at a younger age is increasing. Thus, about one third of patients are under the age 60 and 15-20% are less than 50 years old at diagnosis. This is due to the detection of the disease in an asymptomatic phase on the occasion of blood analysis performed for trivial or routine reasons.

Forms of presentation

Whereas in the 1960s the majority of CLL patients were diagnosed in a symptomatic, advanced phase of the disease, nowadays 80% of the cases are diagnosed when still asymptomatic and in early stage (i.e., Rai 0). This, coupled with the increasingly high number of patients diagnosed at a younger age, poses important treatment problems. Thus, although one of the current paradigms in CLL is that patients in early stage should not be treated, this concept derives from randomized studies performed in the 1960s in which immediate treatment with chlorambucil, with or without corticosteroids, was compared to the same treatment given upon disease progres-
sion. Whether this notion is also valid for newer and more effective treatments is unknown and deserves investigation. The issue of how to treat patients diagnosed on the occasion of routine examinations is further complicated by recent observations indicating that 3.5% of the general population harbors a monoclonal expansion of B-lymphocytes with the characteristic CLL immunophenotype. Of note, such an incidence can be as high as 10% in family members of subjects with CLL. Incidentally, this latter fact should be taken into consideration when selecting sibling donors for allogeneic transplantation.

Overall, only 10-30% of patients are currently diagnosed in the symptomatic phase of the disease, their most common features being lymphadenopathy (20-30%), splenomegaly (15-25%), anemia and/or thrombocytopenia (10-15%). On the other hand, B symptoms (i.e., fever, night sweats, weight loss) are infrequent in CLL patients and should raise the possibility of disease transformation. Occasionally, the first manifestation leading to the diagnosis is autoimmune hemolytic anemia (AHA). Extranodal involvement (e.g., skin, lung, gastrointestinal tract, CNS) is exceedingly rare in CLL.

Evolving patterns

The median survival of patients with CLL has increased from 5-6 years in series reported in the 1970s to 8-10 years in current studies. This is largely due to the higher number of patients who are now being diagnosed in early phases of the disease and, hence, with a better prognosis. Individually, survival, however, is highly variable. Classically, clinical stages have been used to predict the behavior of the disease. Although patients in early stage (i.e., Binet A, Rai 0) usually have a long survival and many of them have a normal lifespan, about 50% of these patients progress at 5 years from diagnosis. Accurate predictors of disease progression include diffuse bone marrow involvement, a rapid lymphocyte doubling time, increased serum markers (e.g., thymidine-kinase, β2-microglobulin, and sCD23), del(11q), del(17p), unmutated IgV genes, and high CD38 expression on neoplastic lymphocytes. Whether patients in an early stage but with poor prognostic signs might benefit from early intervention, before they progress, is unknown; this issue is being investigated in trials. Moreover, prognostic factors should be prospectively assessed since as new, more effective ones become available prognostic factors may change.

Whereas disease progression is the rule in the majority of cases, it is worth noting that around 1% of the patients may undergo spontaneous remission of the disease, frequently after suffering from severe viral infections; this indicates the importance of immunosurveillance mechanisms in the control of the disease and points to potential new treatment modalities aimed at enhancing immunosurveillance.

Disease transformation and other complications

About 10-15% of patients undergo disease transformation into large cell lymphoma (Richter’s syndrome) or, more rarely, Hodgkin’s disease. Signs to suspect disease transformation include the enlargement of lymphadenopathy, the appearance of B-symptoms, increasing serum LDH levels, hypercalcemia, or detection of a serum M component. 67Ga scans show increased uptake in some, but not all, of these cases. Diagnosing disease transformation is important since the treatment in such a situation is that for aggressive lymphoma and, unless a response is achieved, the prognosis is poor with a median survival of less than 2 years after transformation occurs.

Hypogammaglobulinemia is observed in 20-30% of the cases and is considered to be the main cause of infections. Immunoglobulin replacement does not modify life expectancy although it may improve quality of life by decreasing the number of infections.

Moreover, 15-30% of the patients develop either a positive Coombs’ test or clinically overt AHA. AHA can be triggered by treatment and it appears to be more frequent in patients who receive fludarabine. Whether fludarabine should be spared in patients with a positive Coombs test or AHA is an unsolved issue, although this is the practice followed in most institutions. Other immune complications, although rare, are immune thrombocytopenia (<5%) and pure red-cell aplasia (1%). Corticosteroids, cyclosporine A, and rituximab are effective treatments for these complications. In cases of AHA refractory to medical therapy, splenectomy could be necessary.

The incidence of second cancers in individuals with CLL is increased (around 5%), the most frequently involved organs being skin, lung, gastrointestinal tract, and the CNS. Therefore, any new symptom not easily explained by the disease should raise the possibility of a second cancer or, as discussed above, disease transformation. Myelodysplastic syndrome and secondary acute myeloblastic leukemia are rarely reported in CLL patients but their incidence might be increasing due to the use of more intensive treatment approaches.
Conclusions

CLL is a complex and heterogeneous disease. Given the lack of a truly curative treatment and the variable impact of the disease on patients’ survival, risk-adapted therapies are required. The diagnosis of the disease is increasingly being made in younger individuals with indolent disease. The concept that patients in early stage should not be treated should be re-challenged as new and more effective treatments for this disease are now available. At the same time, a large proportion of patients are older than 70 at diagnosis. Since these patients are usually too fragile to tolerate intensive treatments, specific treatment modalities for these patients need to be developed. CLL is also a unique disease in the sense that immune defects are an important component of it. These abnormalities should also be taken into consideration when planning treatment. Other factors to be kept in mind are the possibility of disease transformation into more aggressive lymphoproliferative disorders and the increased risk that CLL patients have of contracting second cancers.

References

The clinical course of patients with chronic lymphocytic leukemia (CLL) is predominantly determined by a profound dysregulation of the immune system. This occurs to such an extent that it has been estimated that almost 60% of deaths are caused by bacterial or viral infections. Together with hypogammaglobulinemia, which is present in a significant percentage of patients, cellular defects leading to impaired immune responses to intracellular bacteria or viral infections, autoimmune disorders and possibly also secondary neoplasms are associated with CLL.

Thus, the intimate interaction of leukemic B-cells with cells of the host immune system is thought to play an important role in the establishment and course of the disease, and several laboratories over many years have focused their attention on defining the abnormalities within the circulating non-malignant immunoregulatory cell populations. This has allowed identification of numerous phenotypic and functional abnormalities occurring in both the T-cell and cytotoxic cell populations of patients with CLL. More recently, attention has been focused on the functional status of the dendritic cell (DC) compartment in these patients, given their pivotal regulatory role on T-cell responses as professional antigen-presenting cells (APCs).

T-cell compartment

Because of the overwhelming prevalence of the malignant B-cell clone, CLL patients show percentages of circulating T-lymphocytes that are much lower than those observed in normal peripheral blood samples, although the absolute number of circulating T-cells is usually increased from the early stages of the disease. Phenotypically, numerous studies have shown an imbalance in the distribution of circulating T-cell subsets, with a relative expansion of the CD8+ T-cell subset and a frequently reduced percentage of CD4+ cells, and a consequent overall significant reduction of the CD4:CD8 ratio. The CD4:CD8 ratio appears to be more severely impaired in patients with a more advanced stage of disease.

Changes in the relative distributions of CD45RA+ and CD45RO+ T-cell subsets and in the expression of HLA-DR have also been reported. The progressive transition from a CD45RA+ CD45RO− to a CD45RA− CD45RO+ phenotypic status has been associated with the generation of an immunologic memory. In CLL patients, increased proportions of CD4+ and CD8+ T-cells express the surface marker CD45RO, suggesting that a significant percentage of CLL T-cells phenotypically resemble activated T-cells. This view is further confirmed by the concomitant increase of HLA class II expression and both abnormalities have been found to correlate with the stage of the disease, being higher in Rai stages II-IV than in stages 0-I.

In addition to the above discussed phenotypic changes, multiple functional abnormalities have been encountered within the residual T-cell compartment of CLL patients. These include a depressed or delayed response to mitogens and antigens, a diminished mixed lymphocyte reaction and a reduced T-lymphocyte colony-forming capacity. Particular attention has focused on the T-helper compartment and decreased activity together with a normal or increased suppressor function were described many years ago. The latter defects are not only a consequence of the reversed CD4:CD8 ratio, but rather are due to a true defect within the CD4+ T-cell subset. Purified CD4 cells from CLL patients do, in fact, show a...
defective helper function towards immunoglobulin (Ig) production by normal lymphocytes, while purified CD8 cells show excessive suppressor activity. These abnormalities, together with the extensive tumor load that impairs the T- and B-cell interconnection, may contribute to the generation of the profound hypogammaglobulinemia frequently observed in CLL patients. More recently, an imbalance has been suggested between Th1 and Th2 immune responses in the T-helper compartment of CLL patients, with a Th2-type response that may progressively dominate over the Th1-type during disease progression. In fact, increased expression of the Th2-associated marker CD30, reduced co-expression of CD7 by CD4+ T-cells and enhanced production of interleukin (IL)-4 have been described in CLL.11,12 De Totero et al.13 reported the expansion, in the peripheral blood of CLL patients, of a CD3+/CD8+/CD28− large granular lymphocyte subset capable of producing and releasing IL-4. This excess of CD30+ CD8 T-cells could represent one of the aspects of the dysregulated CLL T-cell compartment, polarized through a Th2-like immune response.

The activated phenotype of CLL T-cells, combined with their well-known decreased mitogenic and allogeneic responses, suggest that T-cells in CLL patients are in an anergic state. In fact, it has been shown that the T-cell receptor (TCR) ζ chain, which links the TCR to the intracellular signal transduction networks in T-cells, shows a lower expression in CD3+ CLL cells.14 Its expression correlates with the clinical stage of the disease and may normalize after successful treatment. In the same study, the authors also showed a low expression of the CD28 co-stimulatory molecule in both the CD4+ and CD8+ subpopulations, although more marked in CD8+ cells, and it is now known that the lack of co-stimulation through CD28 is the most important factor in the induction of T-cell anergy. Since both molecules are lost in normal T-lymphocytes after prolonged activation with IL-2, it can be suggested that the loss of CD28 and TCR ζ chain in CLL T-cells is a result of a chronic state of incomplete activation in vivo, with consequent induction of an anergic state.

Cytotoxic cell compartment

Numerous abnormalities have been described within the cytotoxic compartment of CLL patients. The overall percentage of circulating natural killer (NK) cells is decreased,14 although several studies have shown that in a proportion of patients there is an increased percentage of CD56+/CD16+ NK cells.15 As for T-cells, individual CLL patients can show increased absolute numbers of NK cells compared to the numbers in normal donors.16 However, a significant decrease in the mean percentage and absolute number of CD16+ cells has been observed in patients with stage C disease compared to those with earlier stage disease.17 Functionally, cytotoxic cells from CLL patients have been shown to be defective in their ability to become activated and lyse appropriate targets. Both NK activity against the K562 cell line and antibody-dependent cell-mediated cytotoxicity (ADCC) are significantly lower than in normal controls.15,18

The normal or elevated numbers of NK cells in CLL patients coupled with decreased NK activity points to the existence of functional defects within the NK compartment. The nature of these defects has not been fully elucidated and is probably worthy of further investigation.

The presence of recurrent infections and of hypogammaglobulinemia has been related to impaired cytotoxic activity in CLL. In particular, it has been reported that CD16+ NK cells from CLL patients with hypogammaglobulinemia, but not from patients with normal serum levels of Ig, are capable of inhibiting mitogen-induced Ig secretion by normal B-cells, although the potential mechanism of action of this suppressive factor has so far not been identified.19 Also, the cell killing by ADCC could be impaired by the reported lack of expression of CD16 (FcγRIII, the low affinity Fcγ receptor) in CLL CD56+ NK cells. Lack of signaling through CD16 may contribute, together with the hypogammaglobulinemia, to decreasing ADCC responses of CLL-derived NK cells and may also preclude their lytic activation and subsequent secretion of cytokines, such as tumor necrosis factor (TNF)-α.20

Dendritic cells

Despite the abundance of information on T-cell defects in CLL patients, very few data are currently available on the status of the DC population in this disease and on its regulatory role on the T-cell compartment. DCs have emerged in the last years as the pivotal initiator and modulator of immune responses, capable of initiating primary T-cell-mediated productive responses as well as of inducing and maintaining T-cell tolerance. Many of the functional defects reported in the T-cell compartment of CLL patients could be related to impaired antigen presentation.

In our laboratory, we have attempted a detailed analysis of the circulating DC compartment in CLL patients.21 The results so far obtained point to the
existence of several defects also within this population of professional APCs. In contrast with the findings obtained in normal donors, DCs sorted from peripheral blood of CLL patients appeared as morphologically and phenotypically immature cells, lacking the maturation antigen CD83 and the co-stimulatory molecule CD80, and displaying reduced levels of HLA I and II antigens. Their ability to stimulate allogeneic T-lymphocytes was severely reduced or absent, as was their ability to produce IL-12.

Both the absence of CD80 co-stimulation and the reduced release of IL-12 may impair the ability of DC to prime a Th1 immune response. In fact, when co-cultured in vitro with allogeneic T-cells, DCs from CLL patients could only induce the differentiation of IL-10-producing, Th2-oriented T-lymphocytes. In vivo, these complex alterations, including the almost complete lack of circulating mature DCs in CLL patients and the predominance of a tolerogenic phenotype, might have a peculiar relevance in relationship with the clinical characteristics of the disease. The above mentioned defects reported within the T-cell compartment of CLL patients, the anergic status of Th2-lymphocytes, the prevalence of Th2-type immune responses and the high incidence of infectious complications are all phenomena that could be related to a defective DC population.

DCs can also be generated in vitro from peripheral blood monocytes and used as potent APCs in an attempt to enhance the deficient immune responses in cancer patients, for instance in vaccination protocols. We and others have demonstrated that monocyte-derived DCs from CLL patients, generated in vitro with high doses of cytokines, are functionally normal and capable of inducing a potent allostimulatory effect. Their use in protocols of immunotherapy can thus be envisaged. However, it is worth noting that in our experiments the growth of functional DCs sorted from peripheral blood monocytes and used as potent APCs in an attempt to enhance the deficient immune responses in cancer patients, for instance in vaccination protocols. We and others have demonstrated that monocyte-derived DCs from CLL patients, generated in vitro with high doses of cytokines, are functionally normal and capable of inducing a potent allostimulatory effect. Their use in protocols of immunotherapy can thus be envisaged. However, it is worth noting that in our experiments the growth of functional DCs from peripheral blood of CLL patients appeared as morphologically and phenotypically immature cells, lacking the maturation antigen CD83 and the co-stimulatory molecule CD80, and displaying reduced levels of HLA I and II antigens. Their ability to stimulate allogeneic T-lymphocytes was severely reduced or absent, as was their ability to produce IL-12.

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These multiple phenotypic and functional abnormalities in the T-, cytokotoxic and professional APC compartments of CLL patients are likely to result from the close cell interconnections ongoing between the neoplastic clone and non-neoplastic immune cells. In fact, several pathways of B-T cell interaction capable of influencing the function of non-malignant accessory cell populations and the regulation of immune responses have been described as operational in CLL, both through cytokine production and cell-to-cell contacts. In turn, these may contribute to the progressive immunodeficiency and to the autoimmune complications that characteristically complicate the clinical course of CLL and have an important role in the unfavorable prognosis of a notable number of patients.

References

Chronic lymphocytic leukemia (CLL) derives from the clonal expansion of CD5+ B-cells that are thought to be frozen at a pre-germinal center (GC) or memory differentiation stage. We show here that these leukemic cells contain the hallmarks of ongoing class-switch DNA recombination (CSR) from C\(\mu\) to C\(\gamma\), C\(\alpha\) and/or C\(\epsilon\) genes, including extrachromosomal switch circles, circle transcripts and activation-induced cytidine deaminase. This, together with the transcriptional configuration of the Ig CH locus and the expression of switch-associated protein-70, Ku70, Ku80, DNA-PK catalytic subunit, OCA-B, Rad51, Pms2 and M\(\text{sh}\) transcripts make CLL B-cells phenotypically similar to normal GC B-cells. Actively class-switching CLL B-cells turn off the CSR machinery in vitro unless exposed to exogenous CD40 ligand and interleukin-4. Similar stimuli induce CSR to C\(\gamma\), C\(\alpha\) and C\(\epsilon\) in leukemic cells that do not actively class switch in vivo. These findings complement and extend our recent demonstration that CLL B cells diversified the expressed Ig V(D)J genes in vivo in an ongoing fashion and do so in vitro upon exposure to CD4+ helper T-cells and cross-linking of the B-cell receptor for antigen.\(^1\) Thus, the CLL B-cell clone is not developmentally frozen, but actively differentiates in vivo along a GC-like pathway that includes CSR and, possibly, somatic hypermutation. This differentiation is not driven by an internal neoplastic drive, but is rather elicited by external stimuli provided by the residual normal elements of the immune system.

Funding

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References

High frequency of CD8+ CD28− cells expressing CD30, CD70, and KIR antigens in chronic lymphocytic leukemia may reflect expansion of chronically activated memory cells impaired in immune recognition

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B-cell chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder of relatively mature B-lymphocytes. The evidence of a frequently inverted CD4/CD8 ratio among residual T-cells, together with their defective in vitro helper activity and response to mitogens, has raised questions regarding the potential involvement of T-cells in the pathogenesis and clinical course of CLL. Morphologic and functional abnormalities of the nonmalignant T-cells have been confirmed in patients with B-CLL. Decreased CD40L expression on CLL T-cells and increased production of cytokines inhibiting B-cell apoptosis (interleukin-4, interferon-γ) have been reported, and these findings point to impairment of normal T-B-cell interactions regulating differentiation and immunoglobulin production in the disease. However, in CLL, impressive multiple T-cell clone expansions have been observed, suggesting that T-cells might be selected and amplified to contribute to tumor surveillance. It remains, thus, an open question whether oligoclonal expansions of T-cells may effectively operate in prolonged stability of stage A B-CLL evolution or, on the contrary, represent reactive T-cell subsets which became anergic and unable to mount an efficient immune response as a result of persistent and chronic antigenic stimulation.

We have previously described, in CLL, the expansion of a CD3+CD8+ T-cell subset with unique phenotypic features and capable of releasing interleukin (IL)-4. Following activation, CD30 was strongly expressed on a high proportion of short-term cultured CLL T-cells, as well as on a panel of T-cell clones (TCC), suggesting polarization versus a T-cytotoxic (Tc) phenotype. CD30 was released by activated CLL T-cell cultures and also found in serum samples from CLL patients. We further observed that CD8+CD30+ T-cells in CLL were predominantly CD28−, and these features were also displayed at a clonal level.

It has been recently reported that CD8+CD30+ CD28− T-cells derived from CLL patients are capable of inhibiting CD40L-induced immunoglobulin class switching in non-malignant B-cells through a CD30-CD30L dependent mechanism, suggesting a suppressor role for these cells (Ts2) in normal T-B-cell dialogue and further highlighting that a higher number of CLL CD30+ T-cells could be relevant to the pathogenesis of the disease.

We extend and report here our studies on the expression and function of other molecules critical in mediating interactions with antigen-presenting cells, such as CD27 and CD70, or in inhibiting killing of tumor cells, e.g. killing inhibitory receptors (KIRs).

CD70 and CD27 expression and function on CLL T-cells

CD70 antigen, the ligand of CD27 antigen, is a type II transmembrane protein belonging to a superfamily of ligands (CD30L, CD40L, FasL, etc.) of tumor necrosis factor-receptor molecules. Expression of CD70 is very restricted on normal lymphocytes: absent on resting lymphocytes, it can be induced after activation. It is expressed by a small subset (10%) of activated memory-type peripheral blood B-cells and in tonsils it is positive on the B-cells in a limited number (one out of 10) of germinal centers. CD27 is a type I transmembrane protein, a member of the tumor necrosis (TNF) receptor superfamily (e.g. CD30, CD40, Fas, etc.) expressed on mature thymocytes, on the vast majority of both CD4+ and CD8+ peripheral blood T-lymphocytes, and on a subset of NK and B-cells.

CD27 expression on T-cells is asso-
associated with the helper phenotype (naive T-cells with CD45RA+), whereas most memory T-cells (CD45RA-CD45RO-) lack CD27. The finding that CD70, infrequently found on normal B-cells in vivo, is nevertheless expressed, and often co-expressed with CD27, on B-cells in a great number of CLL cases may further point to dysregulation of CD27/CD70 interactions being relevant to malignant cell growth.

The observation that strong CD30 upregulation on activated CD8+ TLL T-cells is associated with a lack of CD28 expression suggested that these cells could have reached a state of replicative senescence, and prompted us to extend the characterization of these cells in terms of CD27 and CD70 expression. Following phytohemagglutinin activation of purified CLL T-cells we demonstrated a moderate decrease of CD27 antigen and faster kinetics of CD70 expression, compared to in normal controls. We further analyzed CD70 and CD27 expression on a panel of CD3+, TCRαβ+, CD8+ T-cell clones (TCC) derived from 3 CLL patients, the same showing CD30 positivity. All the 16 TCC studied expressed CD70 and 7 co-expressed CD27. In contrast CD3+, TCRαβ+, CD8+ TCC derived from healthy donors were mostly CD27+ but CD70-. Moreover all the TCC expressed CD45RO and some also co-expressed CD45RA, further indicating an activated state of these cells.

Expression of KIRs on T and NK CLL cells

Upon binding to MHC class I molecules on target cells, KIR antigens (KIRs) deliver a negative signal that prevents CTL- and NK-mediated lysis and their expression may thus affect an efficient antitumor response in a cancer-bearing host. KIRs are predominantly expressed by T-cells with a memory phenotype (CD45RO+, CD28-, CD29+, CD18+), are characterized by a skewed TCRVβ repertoire and are oligoclonal or monoclonal. Two molecularly distinct KIR families have been identified: i) the Ig superfamily, including p58.1, p58.2, p70, and p140 molecules and ii) the C-type II lectin superfamily, including the CD94-NKG2A receptor complex. With reference to our observations that a large fraction of CD8+ T-cells in CLL display features of chronically activated memory T-cells (CD45RO+CD70+, CD28-), we investigated expression of KIRs on T- and NK-cells. Within the panel of CD3+CD8+ TCC previously described, we found a high frequency of CD94 expression. Interestingly, when we studied, at a bulk level, CLL cells depleted of B-cells, by cytofluorimetric analysis with specific moAbs, we found high CD94 and p58.2 expression in 7 out of 16 CLL patients studied. All these patients had stage A disease, were typed as CD38-, and had a percentage of non-B-lymphocytes between 15-30% and a CD4/CD8 ratio ≤ 1.

Furthermore one of these patients (BB), with stable disease and no phenotypic changes for almost 8 years, also showed an increased percentage of NK-cells (CD2~/CD3+/CD16+), and p58.2 and p70/p140 KIRs expression, both on CD8~/CD16+ and on CD8+/CD16+ cells. CLL cells depleted of B only (Tb-) or of B and CD4+ cells (Tb-T4-), after 2 days IL2 in vitro activation, showed 100% cytotoxicity against the K562 cell line. In a redirected killing assay against the P815 cell line, however, only the fraction depleted of CD4+ T-cells resulted cytotoxic by the use of anti-CD3, CD16, p58.2, p70/p140 moAbs. This preliminary observation may indicate that IL2-activated CD4+ T-cells could influence cytotoxicity of NK+ and CD8+ T-cells in this patient.

Conclusions

We have here reported evidence of an expanded subpopulation of CD3+/CD8+ T-cells with features of chronically activated cells, being CD30+, CD70+, CD45RO+ and CD28-. These cells, interestingly, secrete IL4 and IFNγ but not IL2, suggesting a shift versus a Tc2 phenotype that may favor abnormal growth of the malignant B-cell clone. The findings of CD30L and of CD27/CD70 expression on neoplastic CLL B-cells, together with the high frequency of CD30+ and CD70+ on the T-cell counterpart, appear to be relevant to a potential dysregulation of interactions between ligands and receptors that may lead to impairment of Ig secretion and T-cell activation during antigen-specific immune responses. Faster kinetics of CD70 expression and higher CD70 positivity on CLL CD8+ T-cells than on normal controls further support the concept that these cells represent expansions of memory cells after antigenic encounter. However, it is possible to hypothesize that a persistent antigenic stimulation, the dysregulation of normal T/B and T/T interactions regulating cell differentiation or the presence of suppressor subsets may have later
affected their competence in immune responses. The observation of an increased frequency of KIR expression deserves further consideration in the context of signals transduced by these cells that may negatively regulate potential cytotoxic activity against malignant B-cells. Future investigations will be undertaken to establish clearly the role played by these peculiar subsets of CD8+ and NK+ cells in CLL.

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Is CLL one disease?

Chronic lymphocytic leukemia: a proliferation of B cells with distinct genetic and phenotypic features

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B cell type chronic lymphocytic leukemia (B-CLL) is one of the most prevalent leukemias. This leukemia arises from mature B cells that express surface membrane CD5 in the majority of the cases. However, there is controversy about whether there are one or two different B cell subsets that give rise to B-CLL, whether these cells are at the same or different stages of maturation at the time of their transformation, and whether the same type of differentiation pathway is followed by all B-CLL cell precursors.

The expression of IgD and CD38 has been used to identify B cells at various stages of activation and differentiation. Figure 1 schematically depicts the presumed phenotypic profiles that accompany the process of B cell differentiation triggered by antigen, in either a T-dependent or T-independent manner. A characteristic feature of B cells that have undergone a T-dependent maturation process is the presence of Ig V gene mutations.

In this regard, studies by our group and others have provided evidence that ~50% of B-CLL cases arise by transformation of a B cell that has accumulated somatic mutations. Since the remainder of B-CLL cases does not display mutations, this property can be used as a means of identifying subgroups of B-CLL patients.

This distinction led to several hypotheses about the types of cells from which this leukemia derives. In one scenario, the leukemic cells arise from two distinct B cell subsets: one that expresses Ig V gene mutations and is representative of post-GC memory B cells, and another that is antigen naive and does not express V gene mutations. Another scenario is that the B-CLL subgroups derive from conventional B cells stimulated by either T dependent or T-independent responses. A third hypothesis is that B-CLL cells derive from a single cell type that was triggered T-independently and that led to either mutated or unmutated Ig V gene.

In an attempt to address this question, we analyzed the potential role of antigen drive in this disease by studying: [1] the properties of the Ig genes expressed in these cells, [2] their state of activation as reflected by the expression of surface membrane markers, and [3] their replicative history as assessed by analyses of telomere lengths and telomerase activity. These data suggest that, most B-CLL cases originate from B cells that have encountered and responded to antigen. Presumably differences in the type of antigen encountered or the point in maturation at which transformation occurred determines the observed differences in Ig V gene mutation characteristics of the two types of B-CLL cases.

Characteristics of rearranged Ig VH genes

Ig V gene sequence analyses revealed that V \(_{H} \) 4-34, 3-07, and 1-69 were the most commonly expressed genes in our B-CLL patients. J \(_{H} \) segment use differed among these three genes in that ~90% of the V \(_{H} \) 3-07 genes associated with a J \(_{H} \) 4 segment whereas ~50% of the V \(_{H} \) 1-69 and V \(_{H} \) 4-34 genes associated with a J \(_{H} \) 6 segment. In most instances, D segment use was very similar to that identified in other rearranged V\(_{H} \)D\(_{H} \) genes. However, a large fraction of those cases expressing a 1-69 gene were linked with the D3-3 segment.

The B-CLL cases fell into three groups based on differences in HCDR3 length, amino acid composition, and charge. Each of these varied in a V \(_{H} \) family-related manner. The average HCDR3 length of 1-69-expressing cells was greater than 3-07-expressing B-CLL cells. In most cases, the short HCDR3 segments of the V\(_{H} \)3 group contained a J\(_{H} \)4.
segment, whereas the V_{	ext{h}1} group contained a J_{\text{h6}} or J_{\text{h5}} segment. In addition, V_{\text{h}} 1-69-expressing B-CLL cells frequently contained long stretches of tyrosines coded for by the J_{\text{h6}} segment. These differences in J_{\text{h}} gene association resulted in a V_{\text{h}} family-related hierarchy in charge: V_{\text{h}} 1-69 < 4-34 < 3.07. These differences in HCDR3 may reflect selection for specific structural motifs that facilitate antigen binding, regardless of the presence of V gene mutations.

Expression of activation markers

Lymphocytes can express a variety of surface markers that are indicative of cell adhesion, activation, apoptosis or other properties. When B-CLL cases are segregated based on V gene mutation, higher numbers of cells from the unmutated cases expressed the activation markers CD38 and CD69, whereas more cells from mutated cases showed expression of CD71, CD62L and CD23 (Figure 2). These differences were statistically significant.

Although these surface antigens are expressed at different phases post-activation, their expression by the B-CLL cases with little or no V gene mutations suggests that these cases derive from antigen-activated and not antigen-naive cells. When the expression of CD38 is compared with V gene mutation, a variable degree of exists depending on the study and the experimental approach.

Based on clinical features such as treatment histories and survival post-diagnosis, the patients stratified into two distinct groups. Those patients requiring less chemotherapy and with better prognoses usually displayed mutated IgV genes and/or < 30% CD38-expressing B-CLL cells and those patients requiring more chemotherapy and with much worse prognoses displayed unmutated IgV genes and/or ≥ 30% of CD38+ B-CLL cells.

Analyses of replicative history of B-CLL cells

The lengths of the telomeric ends of chromosomes can be used as a marker of the number of divisions experienced by an individual cell. Thus if B-CLL cells are antigen naive, then they would be expected to have long telomeres with lengths comparable to those of cells that have recently exited the bone marrow. In addition since B-CLL cells do not cycle rapidly but rather survive longer due to an apparent apoptotic defect, the unmutated cases might be expected to have longer telomeres than the mutated cases.

When the lengths of the telomeres of the two types of B-CLL cells are measured and compared with B cells from age-matched healthy donors, the leukemic cells had significantly shorter telomeres than B cells from healthy subjects (Figure 3). This implies a longer replicative history of B-CLL cells compared to normal B cells.

However, when the B-CLL cases were categorized based on V gene mutation or CD38 expression, their telomere lengths differed significantly (p < 0.001). Surprisingly, the unmutated cases or those in which > 30% B-CLL cells expressed CD38 had even shorter telomeres than the mutated cases or the cases with ≤ 30% CD38+ B-CLL cells. These data suggest a more extensive proliferative history for unmutated leukemic cells.

The enzyme telomerase restores telomere loss...
that occurs with each division by adding hexameric repeats (TTAGGG) to the eroding ends of chromosomes. Those patients with unmutated V genes have a higher level of this functional activity in their leukemic cells than the mutated cases.

Collectively, these findings imply that B-CLL cells represent a proliferation of B cells with different evolution histories. Based on the preceding data, it is plausible that antigen plays a role in the process that precedes or involves leukemogenesis. Thus BCR engagement and subsequent cellular activation (if these are the cause for the genetic and phenotypic differences between the cases) can be viewed as a promoting agent for the development of this disease. However, BCR engagement by antigen may also affect the biology of the B-CLL cells after transformation is indeed the BCR signaling pathway remains intact. Studies suggest that B-CLL cells are heterogeneous for this property.

Speculations can be made about the characteristics of the antigen that triggers the B-CLL precursors and possibly the B-CLL cell. Since the most common genes found in the unmutated cases (VH 1-69 and 4-34) can be associated with autoreactivity (anti-IgG/rheumatoid factor activity for 1-69, and anti-RBC or anti-DNA reactivity for 4-34), the notion of ongoing autoantigenic stimulation is tenable. Since autoantigens would not be expected to elicit T cell help in the absence of overt autoimmunity, this type of recurrent autoantigenic drive could lead to uniformly short telomeres in (B-CLL) cells that do not accumulate V gene mutations. Indeed, it has been postulated that the VH 4-34 gene is so inherently autoreactive, regardless of the associated VJL, that its ability to terminally differentiate to Ig-secreting plasma cells is rigidly controlled and prevented, except in situations of gross autoimmunity. In addition, the reactivity of the BCR with alleged (auto)antigens could be a function primarily of the VH gene, with some contribution from the HCDR3.

Concomitant normal cellular processes that would permit the survival of autoreactive B cells or abnormalities in the maintenance of tolerance need to exist to allow these (auto)antigen stimulated clones to persist, which would not be surprising considering the frequency of autoimmune phenomena in B-CLL.
The most obvious reason for thinking that chronic lymphocytic leukemia (CLL) comprises two diseases, similar to each other but distinguishable, is the fact that some patients are killed by it and some are not. Furthermore, this heterogeneity is not a gradual blend from one to the other, but a distinct demarcation. Even more convincing is the fact that the allocation to one camp or the other is predictable from the date of diagnosis.

The surest discriminator is the presence or absence of somatic mutations in the immunoglobulin variable region (IgV) genes. The very fact that most experts used to think of CLL as a tumor of naïve B-cells reinforces this concept. Experts work in tertiary referral centers, but tertiary referral centers see the most serious diseases. CLL is only a serious disease when it has unmutated IgV genes. Schroeder and Dighiero’s observation that at least half of CLLs had somatic mutations came as a surprise to the hematologic community, only slightly surpassed by the report from two groups that patients whose tumors had somatic mutations had a median survival of 25 years compared to eight years for those whose tumors did not. This distinction holds with the accumulation of many more cases in the literature (Figure 1). Unmutated CLL is mainly a tumor of males, but mutated CLL occurs equally commonly in either sex (Figure 2).

The two subtypes differ in their use of IgV heavy chain genes. The S1p1 polymorphism of V1-69 is predominately used by the unmutated subset while V4-34 and V3-23 are almost confined to the mutated subset. These are the commonest genes used but similar biases are found with several other genes. A strange anomaly is the use of the V3-21 gene especially when used with the JH6 gene. In these cases the CDR3 is very short and even those with somatic mutations have a poor prognosis. The two subtypes also differ in their expression of CD38, an activation antigen usually present in the unmutated subtype and much less commonly so in the mutated subset. Karyotypically, the two subsets differ. The mutated subset has either a normal karyotype or deletion at 13q14 whereas the unmutated subset is more likely to have trisomy 12 and deletions at 11q23 or 17p15 are almost confined to the unmutated subset.

There are also physiologic differences in that signaling through IgM (though not IgD) is deficient in the mutated subset but normal in the unmutated subset. The obvious explanation for the difference was the hypothesis that those cases with somatic mutations arose from a cell that had encountered antigen in the context of the germinal center, while those that did not have somatic mutations were derived from a cell that had not encountered antigen. There are reasons for doubting that this is a complete explanation. First, a number of laboratories have questioned the demarcation between mutated and unmutated subsets at 98% homology with the germ line sequence. The level of 98% was chosen because this degree of variance could be caused by the polymorphisms known to be present in these genes, and because other (unknown) polymorphisms were suspected. If diseases with less homology behave badly then the influence of the germinal center becomes questionable.

The truth of this assertion awaits discovery of all the polymorphisms. Investigation of the most appropriate demarcation line demands that deaths unrelated to CLL be discarded from analysis. Patients presenting with advanced disease should also be excluded because an unknown period of asymptomatic disease may have preceded discovery. In our hands the 98% cut-off is still the best (Figure 3).

Second, both subsets express CD27 equally. CD27 is an antigen identified with memory B-cells, suggesting that both subsets have been exposed to
There is something of a circular argument here, because CD27 was originally identified as a marker for memory B-cells because it was found on cells with mutated IgV genes.13

Third, gene expression data from two laboratories14,15 demonstrate that the two subtypes are more similar to each other than to any other B-cell tumor or to any type of normal B-cell, though they are more akin to memory B-cells than any other type of normal cell tested. However, they are distinguishable from each other by the expression and non-expression of several genes.

Accordingly, my current hypothesis is presented in Figure 4.

This hypothesis envisages an intrinsic defect in all CLLs whereby stimulation of the B-cell receptor (BCR) induces a reaction pattern of partial activation, anergy and failure of apoptosis.16 In the mutated subgroup stimulation of the BCR takes place conventionally within the germinal center.
The cell would be destined for apoptosis, were that possible, but instead remains as a slowly accumulating, indolent tumor. In the unmutated subgroup stimulation of the BCR takes place outside the germinal center, whether by T-independent antigen or superantigen. A similar succession of events occurs, but re-stimulation is likely leading to a slow succession of cell divisions. Every extra cell division exposes the cell to further genetic damage. The acquisition of abnormalities such as mutations of ATM or p53 releases the cell from proliferative constraints, leading to a more malignant process.

Finally, the most telling factor is the predictability. IgV gene analysis at diagnosis, that a patient will fall into the benign or malignant subgroup. We have looked at the stage A' described by the French Co-operative Group.

These patients were predicted to have a survival curve similar to that of the general population. It is known, however, that about 25% will progress at 5 years. In our hands IgV gene sequencing provides the most reliable indicator that stage A' cases will progress such as to require treatment (Figure 5).

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References

Is CLL one disease?

B-cell chronic lymphocytic leukemia: pathologic overview

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B-cell chronic lymphocytic leukemia (B-CLL) is a lymphoproliferative disorder deriving from mature B-lymphocytes. It is a rather common type of lymphoid tumor, accounting for the vast majority of chronic leukemias in Western countries and representing 5–7% of all non-Hodgkin’s lymphomas. On nosographic grounds, in the Kiel classification it was identified as an autonomous category among the low grade malignant lymphomas of the lymphocytic subgroup, clearly differentiated from B-cell prolymphocytic leukemia and lymphoplasmacytic/cyttoid lymphoma (immunocytoma) (IC). In the Working Formulation it could be recognized in the A subgroup (low grade, small lymphocytic).

The ever expanding availability of immunohistochemical markers during the last two decades has allowed the acquisition of important data about lymphoma phenotypes. Phenotypic similarities, e.g. CD5 positivity, between some cases of lymphoplasmacytic/plasmacytoid lymphoma and B-CLL have gained importance in the conceptual revision of the two entities. Accordingly, as well highlighted in the REAL classification, B-CLL is now recognized as a slightly wider lymphoma category, than it was in the past classification schemes, and is termed B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (B-CLL/SLL). This category comprises a CD5 positive B-cell lymphoproliferative disorder that can present clinically in a leukemic (B-CLL) or solid (mainly nodal based) (SLL) form.

The cases with features of plasmacytoid differentiation are likely to be those once classified as lymphoplasmacytoid immunocytomas in the Kiel classification; as they actually behave similarly to classical B-CLL/SLL their separation is not justified. The remaining CD5 negative lymphocytic lymphomas of the previous IC category of the Kiel classification, are now identified either as true immunocytomas or as marginal zone B-cell lymphomas. It is worthy of note that the B-CLL/SLL group also includes the B-cell prolymphocytic leukemia (B-PLL) of the REAL classification: this choice was due to the natural tendency of B-CLL to enrich in prolymphocytes with time, eventually ending up with a B-PLL (the so-called prolymphocytoid crisis) and to the fact that the distinction between B-CLL and B-PLL can often be arbitrary, also when the FAB criteria are applied.

In the recently published World Health Organization classification of lymphoid tumors - which largely adopted the structure and principles of the REAL classification - the B-CLL/SLL category differs slightly from the REAL classification, in that B-PLL has been maintained separate.

Consistently with the REAL and WHO classifications, B-CLL/SLL is classified as an indolent lymphoma, meaning that it is a process with a survival measurable in years independently of the administration of drugs. Although the diagnosis is often made on peripheral-blood and/or bone marrow smears, histopathology is fundamental to tumor staging and the assessment of the cytologic aggressiveness and growth pattern. B-CLL/SLL lymphoma is composed of three cell types: the small lymphocyte, with a usually round nucleus with coarse chromatin, only occasionally with an irregular nuclear profile; the pro-lymphocyte, a slightly larger lymphocytoid cell with finer chromatin and wider cytoplasm; and the para-immunoblast, a large lymphoid cell with fine chromatin, an evident central nucleolus and relatively wide, slightly basophilic cytoplasm.

In the lymph node, the histologic appearance and growth of the lymphoma depends on the proportion of the cellular components: when only a small number of the latter two cytotypes are present the growth pattern is diffuse (7%) and densely cellular; when there are higher numbers of prolymphocytes and paraimmunoblasts, they tend to cluster in the so-called proliferation centers or pseudofollicles, associated with a higher mitotic rate and a clear appearance at low magnification: this corresponds to the so-called pseudofollicular growth (85%). The proliferation centers, which are only detectable in tissue sections, are diagnostically extremely useful.
Infact, especially in suboptimally processed samples, when cytologic details are often distorted, the cells can assume an indented nuclear profile raising the problem of the differential diagnosis from mantle cell lymphoma or the neoplastic follicles of follicular lymphomas. When the pseudofollicles enlarge and coalesce together with a prevalent prolymphocytic composition, the so-called tumor-forming growth pattern is realized (8%): the latter was originally described as transition into prolymphocytic leukemia.8

Some cases of the tumor-forming subtype contain large amounts of paraimmunoblasts and correspond to the paraimmunoblastic variant originally described by Pugh et al.;9 at present the last two variants are histologically defined as cases with a prolymphocytoid/paraimmunoblastic evolution.

These evolutions do not seem to be associated with significant phenotypic or molecular changes, except for a higher density of surface immunoglobulins, and were originally thought to represent different and subsequent stages in the natural history of B-CLL. According to the FAB criteria and to several recent publications, B-CLL can be subdivided into typical and atypical variants; the latter, cytologically characterized by atypical morphology of cells in the peripheral blood (namely >10% prolymphocytes or >15% of lymphocytes with cleaved nuclei or lymphoplasmacytoid features) bears a worse prognosis and over time has been associated with unfavorable parameters such as higher lymphocytosis, higher expression of CD23 and CD38 and chromosomal abnormalities (trisomy 12, p53 deletions of loss of heterozygosity). Following this concept, some authors have tried to assess whether nodal histology correlates with prognosis, but unfortunately no consensus has so far been reached.15,16 A perifollicular/marginal zone or interfollicular pattern of growth has also been reported for B-CLL and both at nodal and extranodal sites: such cases, which often only partially involve the organ leaving the lymph node sinuses intact, are difficult to recognize as reported by Gupta et al.18 who found a diagnostic misinterpretation in 13 out of 16 cases. In a publication by Bahler et al.,19,20 such cases were reported and two types of histology were recognized: in the first, proliferating centers are scattered between reactive follicles, in the second they mainly have a perifollicular location. As no phenotypic differences were found compared to classical B-CLL, the authors concluded that these cases can represent early or in situ SLLs also because in one example progressive effacement of the node structure was observed in sequential biopsies. Once the whole lymph node has been involved, it is extremely difficult to distinguish the two patterns of original growth, although those with perifollicular proliferation centers may appear more vaguely nodular.

In the bone marrow the neoplastic cells can infiltrate in nodules or diffusely and display the same cytologic component as in the lymph nodes, while in the spleen, the infiltrate affects both the white and red pulp, although the proliferation centers mainly affect the former.22

Like other indolent B-cell lymphomas, B-CLL/SLL can occasionally associate with a large cell lymphoma: such a condition, commonly known as Richter’s syndrome, can be represented by either a clonally related large B-cell lymphoma or a de novo lymphoma, usually of B-cell type, which has arisen in immunodepressed patients like the B-CLL/SLL ones. However, independently of the clonal relationship, the aggressive B-cell lymphomas tend to retain a B-CLL phenotype (CD5+, CD23+, FMC7+, CD38-).23

As far as phenotype is concerned, B-CLL/SLL expresses B-cell markers, such as CD19, CD22, CD79a and CD20.3,7 The reactivity with CD19 and CD79a is often weak while that of CD20 can show variations among the cell types; in fact, in fixed material, it has been reported24,25 and personally observed that only prolymphocytes and paraimmunoblasts are consistently reactive: although this does not correlate with any other clinicopathologic parameter,24 it can have a clinical impact considering the recent expanding usage of anti-CD20 therapy in the treatment of lymphomas.26

B-CLL/SLL also expresses CD5 and CD23 at membrane level. The detection of such molecules can be difficult on routine biopsies due to variations in the expression density or to the molecules’ high sensitivity to fixation, which can mask their recognition. Regarding the latter point, the application of antigen retrieval techniques27 is thus recommended.

CD23 functions as an IgE receptor and is thought to play a role in the proliferation of B-lymphocytes. It has been reported that its expression is stronger in prolymphocytes and paraimmunoblasts, and that this has a prognostic impact.20 Indeed, this histopathologic observation fits with the adverse significance of high concentrations of soluble CD23 in the serum of B-CLL patients.20,28

CD5 expression is highly specific, even if 7 to 20% of the cases are reported as negative.29 Although some investigators doubt that these forms really exist, regarding them as leukemic non-Hodgkin’s lymphomas, recent data do not show significant clinical differences between the two variants, except...
for a more frequent isolated splenomegaly in the CD5- group.29

The expression of immunoglobulins (Ig) (IgM and IgD) is very weak, so that their detection in tissue section is difficult, except in cases with marked plasmacytoid differentiation in which significant amounts of intracytoplasmic Ig are produced and can be revealed. Additional markers have recently been proposed for the diagnosis and differential diagnosis of B-CLL/SLL, such as cyclin D1, bcl-2 and bcl-6 products, multiple myeloma oncogene 1/interferon regulatory factor-4 (MUM1/IRF4), and PAX-5 gene product/B cell-specific activator protein (PAX5/BSAP).7,31-33

Cyclin D1 is regularly overexpressed in mantle cell lymphoma due to the bcl-1 gene rearrangement or the presence of t(11;14).7,34 However, B-CLL/SLL seldom shows positivity for cyclin D1; this, on the one hand, makes the differential diagnosis even more difficult, especially in cases with suboptimal fixation and distorted cytology or in atypical B-CLLs, and, on the other hand, strengthens the importance of CD23 staining.33

The bcl-2 product is strongly positive in B-CLL/SLL although this is not associated with the t(14;18). The growth fraction, as detected by KI-67 staining, is usually low, although in the proliferation centers slightly higher values can be observed.

cbl2 expression, indicating protection from apoptosis, and the low proliferation index, are consistent with the indolent nature of B-CLL/SLL.

The bcl-6 antigen is usually negative31 or expressed at significantly lower levels than in normal or neoplastic germinal center cells25 and its identification can be helpful in differentiating the pseudofollicles (bcl-6-, CD10-, bcl-2-, CD5+, CD23-) from either residual germinal centers (bcl-6+, CD10+, bcl-2+) or neoplastic follicles of the follicular lymphoma (bcl-6+, CD10+, bcl-2-, CD5-, CD23+).

The antibodies raised against the transcription factors IRF4 and BSAP can also be useful in the diagnosis, giving rise to opposite patterns:22-33 small lymphocytes are BSAP+ and IRF4-, while plasmacytoid and paraimmunoblasts are BSAP+ and IRF4+ as detected by our group in a large series of cases. Considering that IRF4 (product of the MUM-1 gene) is physiologically expressed by B-lymphocytes after selection in the germinal centers (that is, by some centrocytes in the light zone of the germinal center, plasmacytoid elements and plasma cells), our observation fits with the recent hypothesis of a memory B-cell origin of at least some B-CLL cases (see below).22 37-40 MUM1 has also been associated with a shorter survival, although its expression does not correlate with CD38 positivity, IgVH mutational status or any other clinical parameter.41

When considering the phenotype of B-CLL, it is essential to refer to CD38, a surface antigen with multi-functional activity, whose expression has in recent years been considered an independent prognostic marker if expressed in > 30% of the B-cells.

Numerous studies have in fact closely associated CD38 overexpression with recognized unfavorable factors such as atypical morphology, higher peripheral blood lymphocytosis, diffuse bone marrow involvement, trisomy 12, p53 alteration, high soluble CD23 levels, lymphocyte doubling time less than 12 months, higher tumor burden (defined as lymphadenopathy and splenomegaly), Binet B+C stages, Rai III+IV stages and risk of no or partial response to fludarabine.42, 43-44 Due to its pivotal role, CD38 has also been proposed as a surrogate marker of the immunoglobulin heavy chain variable gene status (see below).

Although obvious, it is useful to know that other T-cell markers are negative (although cases with CD8 positivity have been reported in the literature), as well as CD10, CD68, CD72/DBA44.

As far as the differential diagnoses are concerned, B-CLL is usually rather easy to identify due to this peculiar cytologic composition: nonetheless it has to be differentiated from all other small B-cell lymphomas. In this regard the phenotypic algorithm listed above does help, with special reference to CD5 and CD23. Mantle cell lymphoma (MCL) needs to be excluded in cases with slightly indented morphology, which may be either true or induced by suboptimal fixation: its phenotype is CD5-, cyclin D1+, CD10-, CD23+, DBA.44+, CD68-. Follicular lymphoma (FCL) grows in follicles that actually can look like the pseudofollicles of B-CLL and in this sense the phenotypic profile is of aid: FCL is CD5-, CD10+, CD23-, DBA.44-, CD68-, and Bc16 strongly positive.

Immunocytoma is characterized by a prominent plasmacellular differentiation and usually lacks the medium–large sized cells of B-CLL: its phenotype is CD5-, CD10-, CD23+, DBA.44-, CD68-, with monoclonal restriction of light chain immunoglobulin usually at strong intensity.

The marginal-zone lymphoma (MZL) could be a cause of misdiagnosis because of the presence of medium sized cells with a clear appearance, but it usually grows differently and lacks CD5-, although it can express CD23+ (CD10-, DBA.44+, CD68+). Hairy cell leukemia (HCL) is very unlikely to be a source of confusion; nonetheless its phenotype is CD5+, CD10-, CD23+, DBA.44+, CD68+.

On molecular grounds, most data collected in
recent years seem to suggest the existence of two different variants; in fact, it has been observed that about half the cases show unmutated immunoglobulin heavy variable chain (IgVH) genes, while the rest have mutated IgVH genes, with no evidence of ongoing hypermutations: this observation indicates that the unmutated cases have not passed through germinal center antigen-induced selection and could thus derive from naive B-cells, whilst the mutated examples originate from cells that have already escaped the germinal centers after activation, selection and commitment, thus being memory B-cells. Furthermore, the recent evidence of BCL6 mutations only in the IgVH mutated cases confirms these data. The unmutated cases are associated with a poorer prognosis and unfavorable parameters: in this light, since the assay for the determination of IgVH mutational status is rather expensive and the CD38 one highly reliable and cheaper, some recent studies have tried to demonstrate that the latter could be used as a surrogate for the former in the routine definition of prognostic groups and the available results seem to prove them right. In fact, although CD38 does not perfectly overlap with the two subgroups defined by mutational status, it does seem true that the CD38/ unmuted cases have significantly shorter survivals than the CD38/unmutated cases (8 years vs 26 years, respectively), with discordant cases having intermediate median survivals (15 years).

As described in the histology paragraph, no certain prognostic correlations have so far been determined between histology and clinical evolution. To this regard, some interesting data emerged from the publication by Bahler et al., about cases of interfollicular SLL. In fact, the molecular studies performed showed that 3/5 cases with perifollicular proliferation centers had highly mutated Vh genes, whereas the majority (5/6) of the cases with proliferation centers located between reactive follicles were unmutated. These results, while shedding light on the possible impact of histology upon prognosis, strengthen the importance of the search for Ig mutational status on routine material.

The recognition of two molecularly different forms of B-CLL leads to the thought that also the cell of origin could be different, further complicating the enigma on the normal counterpart of B-CLL.

References
18. Gupta D, Lim MS, Medeiros LJ, Elenitoba-Johnson KS. Small lymphocytic lymphoma with perifollicular, marginal zone, or interfollicular distribution. Mod Pathol 2000;


Chronic lymphocytic leukemia (CLL), the most frequent adult leukemia in the Western world, is a neoplastic disease of advancing age, characterized by a progressive accumulation of functionally incompetent, long-lived small mature monoclonal B-lymphocytes, with a characteristic phenotype (CD5+, CD23+, low surface immunoglobulins and CD79b). It is far from uniform in presentation and clinical course. About one-third of patients never require treatment and have a long survival; in another third an initial indolent phase is followed by progression of the disease; the remaining third of patients have aggressive disease at the outset and need immediate treatment.

The development of the Rai and Binet staging systems has allowed the division of patients with chronic lymphocytic leukemia into three prognostic groups: good, intermediate and poor prognosis (Table 1). Binet’s good prognosis group (stage A, 63% of CLL patients with a 10-year survival of 51%) includes twice as many patients as Rai stage 0, since it includes all Rai stage 0, 2/3 of Rai stage I and 1/3 of Rai stage II. Rai stage 0 patients, who include 31% of CLL patients, display a 10-year survival of 59%. Rai’s intermediate prognosis group includes 59% of CLL patients compared to 30% in Binet’s intermediate group. These differences can affect the design of clinical trials.

The two staging systems have improved the identification of patients who need immediate treatment. Two long-term French trials and a meta-analysis of most randomized trials demonstrated that therapy with chlorambucil, an oral alkylating agent and the standard treatment of chronic lymphocytic leukemia, could be deferred for Binet’s stage A patients. This low-risk group, which constitutes almost two-thirds of patients with chronic lymphocytic leukemia, has a median age at diagnosis of 64 years and an expected survival of >10 years, which is close to the life expectancy of a normal population matched for sex and age. Moreover, deferring therapy until forced by disease progression does not compromise survival. However, as shown in Table 1 over 25% of these indolent cases die of causes related to chronic lymphocytic leukemia, 40% progress to advanced stages, and 50% ultimately require treatment.

Neither the Rai nor the Binet staging system can predict which patients among the good prognosis group will shift into progressive disease and there is heterogeneity among stage B and C patients. Lymphocyte doubling time, serum levels of β2-microglobulin, thymidine kinase and soluble CD23 as well as CD38 expression on malignant cells can help predict disease activity, but the presence in the leukemic B-cells of cytogenetic abnormalities such as 11q deletions or somatic mutations in the immunoglobulin heavy chain genes are better predictors of rapid progression and survival. To define these issues we compared the prognostic value of Ig mutational status within the different stages of the Binet staging system in 146 patients.

In addition, since sequencing of IgVH genes is not available in most laboratories and an easily performed surrogate assay is desirable, we examined the ability of sTK, sCD23, β2-microglobulin and CD38 to predict the mutational status of Ig V\textsubscript{H} genes.

Based on the V\textsubscript{H} gene status, our series consisted of 80 unmutated and 66 mutated cases. The Binet staging system revealed a heterogeneous distribution, with predominance of A patients in...
the mutated (75%), and B/C in the unmutated (69%) group. The median follow-up of this population was 61 months (range 1-432). Prognostic value was assessed in terms of overall survival and progression-free survival. Unmutated (UM) cases displayed a median overall survival (OS) and progression-free survival (PFS) of, respectively, 84 and 68 months, while for mutated (MT) ones the median OS was not achieved (70% 12-year survival, p < 0.0001) and PFS was 141 months (p < 0.0001).

Regarding stage A patients, median OS and PFS were significantly shorter for UM than MT cases (97 months vs not achieved, p = 0.0017; and 42 vs 156 months, p < 0.0001 respectively). Hence, compared to the previous A’ and A” substaging, the Ig VH status of stage A cases advantageously predicted prognosis. Interestingly, the mutational status was also able to segregate stage B and C patients into two groups with different survival patterns (median OS of 78 vs 120 months for UM and MT cases, respectively; p = 0.002). Remarkably, no death was observed among the 7 stage C patients with a mutated profile.

As concerns the ability of sTK, sCD23 and CD38 (complete data on these 3 parameters were available for 57 patients) to predict the mutational status of Ig V\textsubscript{\textalpha} genes, our results demonstrate that serologic levels of TK and CD23 can reasonably predict the mutational status of Ig V\textsubscript{\textalpha} genes (p = 0.03).

Our results clearly demonstrate that the mutational status of Ig V\textsubscript{\textalpha} genes is the best prognostic indicator in CLL within all Binet stages. Since mutational status of Ig V\textsubscript{\textalpha} genes is still a time consuming and highly specialized technique, sCD23 and sTK, may in the near future constitute reasonable surrogate markers for this technique.

These recent studies on Ig V genes may suggest that there are two types of chronic lymphocytic leukemia according to the mutational pattern of Ig V genes: one arises from relatively less differentiated (immunologically naive) B-cells with unmutated heavy chain genes and has a poor prognosis; the other evolves from more differentiated B-cells (memory B-cells) with somatically mutated heavy chain genes and has a good prognosis.\textsuperscript{10-12}

However, recent data derived from gene expression profiling analysis failed to distinguish unmutated and mutated cases clearly and favor the view that all cases of CLL have a common cell origin and/or a common mechanism of malignant transformation.\textsuperscript{13,14}

To examine this issue, we used microarray technology to determine whether gene expression profiles could distinguish these two groups of CLL.

Eighteen cases of CLL, including 9 Binet stage A cases with stable disease (i.e. at least 5 years without treatment and any evolution) and mutated Ig genes, and 9 cases with stage B or C aggressive disease and unmutated Ig genes, were studied. For these latter cases, only cells collected before therapy were analyzed. Leukemic cells were purified by negative selection, yielding > 98% pure CLL cell populations. RNA was extracted and gene expression analyzed using the Affymetrix human U133 Genechip with 22283 probe sets.

In agreement with previous reports indicating that Ig mutated and unmutated cases globally have the same gene expression pattern, a supervised statistical analysis showed that only 85 genes were differently expressed by a factor > 2 between the two groups of CLL. However, in contrast to previous reports, a non-supervised hierarchical clustering analysis clearly separated the stable mutated group from the aggressive unmutated one, except for one case.

These results show that gene expression profiling can distinguish CLL cases with a stable evolution and mutated Ig genes from those with unmutated Ig genes and progressive disease. Thus, monitoring the expression of a very limited number of genes might suffice to identify patients with an indolent disease from patients exhibiting an aggressive one.

Table 1. Rai and Binet good prognosis patients after 11 years’ evolution (results from the CLL-80 study).

<table>
<thead>
<tr>
<th>Stage</th>
<th>% of pts.</th>
<th>10-year survival</th>
<th>% of pts without evolution</th>
<th>% of pts related deaths</th>
<th>% of pts evolving to B or C</th>
<th>% of pts receiving treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage A</td>
<td>65%</td>
<td>51%</td>
<td>47%</td>
<td>31%</td>
<td>41%</td>
<td>53%</td>
</tr>
<tr>
<td>Stage B</td>
<td>31%</td>
<td>59%</td>
<td>57%</td>
<td>27%</td>
<td>32%</td>
<td>43%</td>
</tr>
</tbody>
</table>

References

Chronic lymphocytic leukemia: is it a curable disease?


Different Therapies for Different Diseases?

B-cell chronic lymphocytic leukemia: different therapies for different diseases?

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The well-known heterogeneity of B-cell chronic lymphocytic leukemia (B-CLL) on the one hand and, on the other hand, the expansion of therapeutic options for indolent lymphoproliferative disorders raise the question of possible different therapeutic choices based on the clinical and biological features of each patient. For decades the problem of the biological heterogeneity of B-CLL has been fueled by considering hematologic, morphologic and immunophenotypic parameters in studies aimed not only at defining the precise diagnosis (CLL versus PLL versus non-Hodgkin’s lymphoma with leukemic expression), better but also at identifying patients with different prognoses.

So far, controversial results have emerged, generally not able to give sufficient information useful to guiding therapeutic choice. However, in the last few years two major advances in B-CLL biology have been made and it is hoped that these will enable clinicians to identify different diseases within the B-cell chronic lymphoproliferative disorders suitable for differentiated therapeutic plans. I do, of course, refer to: 1) the identification of IgV gene status; 2) the possibility of successfully performing cytogenetic studies in the majority of B-CLL patients, because of the development of molecular cytogenetics by fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH) techniques.

Biological aspects

It has now been clearly established that IgV gene status (mutated versus unmutated) identifies two groups of B-CLL patients with different prognoses.2,3 Beside the biological importance of this finding that opens new horizons on the issue of the cell originating B-CLL, it is well demonstrated that the unmutated IgV gene configuration is associated with an unfavorable clinical outcome.

The major obstacle to systematic and widespread use of this marker as a prognostic parameter is that, so far, the technique is expensive and limited to very specialized laboratories, thus not easily extended to the many institutions involved in the diagnosis and treatment of B-CLL, which, as everybody knows, is the most common leukemia in Western countries. In order to overcome this obstacle, simplification of the technique or introduction of an easier surrogate marker of IgV gene configuration is necessary.

Apparently, a good, easy surrogate marker could be the expression of CD38 antigen, whose higher expression was initially reported as associated with the IgV unmutated configuration.2,4 Unfortunately, the association of these two markers is still controversial,3 such that, so far, high CD38 expression can only be considered a predictor of a worse prognosis, not invariably associated with IgV gene configuration, and may change during the course of the disease.

Another recent biological advance in B-CLL is the development of molecular cytogenetic techniques capable of studying virtually all patients successfully. From studies on large series many cytogenetic aberrations have been found to be associated with different clinical outcomes. Again in this case, technical aspects so far hamper their application to the majority of B-CLL patients.

An updated list of prognostic parameters is reported in Table 1.

Therapeutic options

The possibility of treating different B-CLL diseases with different therapies stems from the availabili-
Chronic lymphocytic leukemia: is it a curable disease?

Each option deserves some comments:

**Watch and wait policy** (observation without therapy). This option is considered the appropriate management for patients with early and stable disease. However, this conclusion was drawn from studies performed in a pre-fludarabine and pre-monomoclonal antibody immunotherapy era. Moreover, the definition of an early, stable phase of the disease was based on clinical and hematologic parameters, while biological parameters, better defining a high probability of progression, should now be used, especially in younger patients.

In other words, new studies are warranted taking into consideration new biological features and new treatments.

**Conventional therapy for palliation**. This option includes all therapies aimed at controlling the disease with minimal toxicity using agents known to be safe but unable to induce a high quality of response. These therapies include low-dose alkylating drugs (chlorambucil and cyclophosphamide) and fludarabine as a single agent.

**Aggressive conventional therapy**. It is well demonstrated that high-dose continuous chlorambucil therapy can induce a high response rate with long response duration and survival; this approach has the important advantage of costing less than other treatments.

**High-dose chemotherapyy with stem cell rescue**. B-CLL is a hematologic neoplastic disease in which the transplant approach started just a few years ago. According to the results so far reported high-dose continuous chlorambucil therapy can induce a high response rate with a long time to progression, while eradication of the disease is very rare.

**Immunotherapy**. At the moment numerous reports deal with the use of monoclonal antibody therapy, namely anti-CD20 and anti-CD52, in B-CLL. From the reported experiences it appears that their
activity, in terms of both response rate and response duration, is rather limited when used as single agents. In contrast, their combination with fludarabine and other drugs is able to elicit a higher response rate of better quality and duration.

Treatment planning

It is now commonly accepted that B-CLL patients should be treated according to a risk-adapted model. On this ground the choice of the appropriate treatment for each single patient has recently become more difficult than ever. In fact, the great number of therapeutic options, not fully analyzed for their long-term efficacy, and, at the same time, the recent advances in biological findings make it difficult to decide the very best treatment in individual patients. On the other hand, we have to take into consideration the cost of many new treatments and the laboratory examinations aimed at better defining biological prognostic features.

Thus, keeping in mind that we are in a rapidly developing field, a tentative plan for the treatment of B-CLL has been designed (Figure 1).

According to this proposal, patients are treated differently according to their age and risk features at presentation; these risk features include common parameters, such as clinical stage, and new biological markers, such as IgV gene mutation, cytogenetic alterations, CD38 expression and, not less important parameters reflecting cellular growth such as serum TK and β2 microglobulin.

Conclusions

For the time being, the important message is that the scenario of B-CLL therapy is changing drastically. In the next few years we should be able to achieve optimal use of the several new treatment options in their appropriate setting, thus avoiding a waste of relevant resources and obtaining a further improvement in the prognosis of B-CLL.

References

After some years of stagnation, chronic lymphocytic leukemia (CLL) has entered center stage in hematology and oncology. Starting with the era of purine analogs, the last fifteen years have brought a dynamic development of new investigative compounds for this disease such as never experienced before in the history of hematology. In addition, monoclonal antibodies such as rituximab and alemtuzumab have become available. New prognostic parameters such as chromosomal deletions 17p- or 11q-, assessed by molecular cytogenetics, an unmutated immunoglobulin V\textsubscript{H} gene, serum parameters (p2-microglobulin, thymidine kinase, CD23), and the assessment of disease activity all allow a more precise prediction of the prognosis of an individual patient, independently of the Binet or Rai stage. Given this wealth of new diagnostic and therapeutic modalities, we are now challenged to combine these tools in a new, optimized strategy of CLL management during the next decades. In this regard, the combination of chemotherapy with monoclonal antibodies (chemo-immunotherapy) is currently one of the most attractive options. It is obvious that prospective, randomized trials are needed to evaluate these new treatment modalities. Towards this end, the GCLLSG has defined a risk- and age-adapted strategy for the first-line treatment of CLL, with 6 phase III trials currently underway (Figure 1).

Management of early CLL (Binet stage A)

The CLL1 protocol attempts to define the role of early use of fludarabine in early stage, high-risk CLL. High risk of disease progression is defined as elevated β2-microglobulin OR elevated serum thymidine kinase levels AND lymphocyte doubling time shorter than 12 months OR non-nodular bone marrow infiltration. High-risk patients comprise approximately one third of all cases with early CLL. These high-risk patients are randomized to receive either fludarabine or no treatment, while low-risk patients do not receive any treatment. The protocol has now recruited more than 600 patients and will be closed shortly. The next trial of the GCLLSG on early stage CLL (CLL7 protocol) will evaluate the use of chemo-immunotherapy, consisting of fludarabine, cyclophosphamide and rituximab, versus no treatment in patients at high risk (as defined by molecular and serum markers).

Management of advanced CLL (symptomatic Binet stage B, all patients with Binet stage C)

These patients usually require treatment. The GCLLSG has defined the following treatment goals:
1. To achieve long-lasting remissions (cure?) in younger patients (up to 65 years).
2. To achieve optimal palliation in older patients (over 65 years).

This strategy is reflected by the following active trials:
1. For younger patients (up to 65 years), an intensified strategy of chemo-immunotherapy is being evaluated. Patients receive fludarabine (F) versus fludarabine/cyclophosphamide (FC) followed by a post-remission consolidation with alemtuzumab versus no treatment (CLL4 and CLL4B protocols).
2. Patients older than 65 years receive fludarabine versus intermittent chlorambucil (CLL5 protocol).

The next trial of the GCLLSG for patients who are less than 65 years old (CLL8 protocol) will investigate FC plus rituximab versus FC alone and assess the frequency of molecular remissions achieved by this treatment.
Patients at relapse
Patients at relapse are included into the CLL6 protocol testing the use of granulocyte colony-stimulating factor (G-CSF) for the prevention of infections in patients treated with the triple combination of fludarabine, cyclophosphamide and mitoxantrone. In addition, phase II studies are active to evaluate new treatment modalities such as fludarabine plus alemtuzumab (CLL2H protocol) or CDaXOPR (CLL2G protocol: cyclophosphamide, vincristine, liposomal daunorubicin, prednisone, rituximab).

High-dose therapy
The GCLLSG has developed experimental high dose therapy protocols for first or second-line treatment. It must be pointed out that only a minority of CLL patients is eligible for these protocols, because of advanced age and concomitant diseases, or the absence of appropriate risk factors justifying intensive therapeutic measures.

The high-dose therapy is followed by autologous or allogeneic progenitor cell transplantation (CLL3C, CLL3X and CLL3R protocols). In the CLL3C and CLL3X protocols, chemo-immunotherapy, consisting of FC plus alemtuzumab, is used as cytoreductive treatment prior to stem cell harvest or allogeneic transplant.

Conclusion
The GCLLSG has constructed a risk- and age-adapted treatment strategy. The overall goal of these trials is to improve the outcome (survival) of CLL patients by maintaining an optimal quality of life. It cannot be stressed enough that co-operative, randomized trials are the only way to proceed reliably towards these goals.
The Role of:  
2-chlorodeoxyadenosine in the treatment of B-cell chronic lymphocytic leukemia

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The compound 2-chlorodeoxyadenosine (2-CdA, cladribine) is the drug of choice for the treatment of hairy cell leukemia, but it is also highly active in other low grade lymphoid malignancies including chronic lymphocytic leukemia (CLL). 2-CdA is a deoxyadenosine analog with a substituted halogen atom at position 2 in its purine ring that makes it resistant to deamination by adenosine deaminase (ADA). 2-CdA is phosphorylated by deoxycytidine kinase and accumulated as chlorodeoxyadenosine triphosphate (2-CdATP). High activity of this enzyme in lymphocytes along with their low 5-nucleotidase activity is the probable explanation for 2-CdA’s relatively high selectivity for these cells.1-3 This metabolite disrupts cell metabolism by incorporating into the DNA of actively dividing cells, including DNA single-strand breaks, and inhibiting DNA synthesis. Moreover, 2-CdA inhibits ribonucleotide reductase and leads to an imbalance of deoxyribonucleotides in the intra-cellular pool. In vitro studies have also shown that 2-CdA increases the level of apoptosis of B-CLL cells. Recent laboratory studies suggest that 2-CdA can induce CLL cell death by direct mitochondrial injury in a way that is different from the intrinsic caspase-9 apoptotic pathway initiated by fludarabine (FAMP).7

The drug is routinely administered intravenously. However, the best method for 2-CdA administration remains to be determined. Santana et al. have shown that continuous intravenous infusion of this agent would produce greater cell death.4 However, the report of Liliemark and Julliusson indicates a terminal half-life of 6.3 hours after a 2-hour infusion of 2-CdA, which may permit the drug to be administered as an intermittent infusion without loss of antitumor activity.5

On the basis of this study we administered the drug at a dose of 0.12 mg/kg daily in 2-h intravenous infusions for 5 consecutive days in the majority of our studies.6-9 This route of administration is free from the inconvenience of catheterization of veins usually associated with more frequent thrombotic complications. Moreover, 2-h infusion is more convenient and may be given on an out-patient basis. 2-CdA can also be administered in subcutaneous injections and orally.10,11 These routes result in substantial improvement of the quality of life in disorders that require repeated courses of treatment, such as CLL. Oral administration of 2-CdA has a bioavailability of approximately 50%,10 thus the oral dose of 10 mg/m² corresponds to 5 mg/m² by the intravenous or the subcutaneous route.10,11

2-CdA in pre-treated patients with CLL

The activity of 2-CdA in patients with CLL resistant to conventional treatment was first reported by Piro et al. in 1988.12 An overall response was achieved in 10 out of 18 patients. The same group later reported a response rate of 44% in 90 patients with advanced and previously treated CLL.13 However, only 4% of the patients achieved CR. In this study, the course of 2-CdA consisted of 0.05 to 0.2 mg/kg/d for 7 days by continuous infusion. In our retrospective analysis of 184 patients with CLL in relapse or refractory to previous therapy we found an overall response rate of 48.4%, including 12.5% CR.9 2-CdA was administered at a dose of 0.12 mg/kg daily by 2-h intravenous infusions for 5 consecutive days. Similar results were achieved by Betticher et al. when the drug was administered subcutaneously.13 In this study patients were treated with 2-CdA given at a reduced dose of 0.5 mg/kg/ cycle as s.c. bolus injections for five days. The overall response rate was 40% and was similar to that in a group of 20 patients treated with 2-CdA at a dose of 0.7 mg/kg/cycle as continuous i.v. infusion for 7 days.
Moreover, dose reduction by 29% resulted in significant decreases of myelotoxicity and risk of infection. Similar results have been reported by others (Table 1).6,13-17 Despite the high response rate, the influence of 2-CdA on survival duration in CLL is still uncertain. However, in our retrospective analysis we found that patients who received high dose chlorambucil with prednisone as a front-line therapy followed by 2-CdA with or without prednisone as a second-line treatment survived significantly longer than the patients never treated with 2-CdA.18 It should be underlined that the difference in survival was seen only in patients with more advanced clinical stages of CLL (Rai III and IV), whereas in less advanced stages (Rai 0, I, II) survival was similar in both groups (Figure 1).

### Table 1. 2-CdA in previously treated patients with CLL.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of patients</th>
<th>OR (%)</th>
<th>CR (%)</th>
<th>Median duration of response (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saven14</td>
<td>90</td>
<td>44</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Tallman15</td>
<td>26</td>
<td>31</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Juliusson16</td>
<td>52</td>
<td>58</td>
<td>31</td>
<td>20</td>
</tr>
<tr>
<td>Robak6</td>
<td>184</td>
<td>48.8</td>
<td>12.5</td>
<td>10</td>
</tr>
<tr>
<td>Rondelli17</td>
<td>19</td>
<td>68</td>
<td>11</td>
<td>CR, 9+</td>
</tr>
<tr>
<td>Betticher13</td>
<td>55</td>
<td>38</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

CR: complete response; OR: overall response.

2-CdA in front-line therapy

There is less experience with the use of 2-CdA than with fludarabine (FAMP) in patients with CLL in the majority of Western countries. Similarly to FAMP, this agent has been found to be more effective in previously untreated CLL than in patients refractory to or relapsed after conventional therapy with alkylating agents. In different studies the overall response rate ranged from 75 to 85% and CR from 10 to 47% (Table 2).6,7,11-19,24 In our recently updated phase II study on 184 previously untreated patients with B-CLL, CR was observed in 88 (45.4%) patients and PR in 72 (37.1%), for an overall response rate of 82.5%.6 The median duration of OR (CR or PR) in this group of patients was 12.0 months and that of CR 13 months. The median survival was longer in patients who responded to 2-CdA treatment than in non-responders.

High CR and OR rates in CLL patients treated with 2-CdA as a first-line therapy were confirmed in a multicenter, prospective, randomized trial. In this study we compared the efficacy and toxicity of 2-CdA with prednisone and chlorambucil with prednisone in previously untreated patients with progressive and advanced B-CLL.7 2-CdA was administered at a dose of 0.12 mg/kg daily in 2h infusion for 5 consecutive days and was combined with oral prednisone 30 mg/m² daily on days 1 to 5 starting with 2-CdA courses. Chlorambucil was administered at a dose of 12 mg/m² per day for 7 consecutive days and prednisone was given at a dose of 30

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Figure 1. Survival of patients treated with high-dose (HD) chlorambucil (Chl) plus prednisone (P) as a first-line and 2-CdA±P as a second-line treatment in comparison with patients treated with standard dose of Chl+P as a first line and COP/CHOP as a second-line therapy. Survival was measured from the beginning of first treatment and the analysis was done using the method of Kaplan and Meier. The log-rank test was used to compare treatment groups. (A) Survival of early stage patients (Rai 0, I and II) and (B) late stage (Rai III and IV) (Modified from Robak T, Blonski JZ, Kasznicki M. Does intensive treatment with high dose chlorambucil and prednisone as first line and cladribine as second line influence the survival of the patients with chronic lymphocytic leukemia? Leuk Lymphoma 2001; 41:545-57). With kind permission of the Publisher.
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mg/m² per day on days 1 to 7. Both cycles were repeated at monthly intervals or longer if hematologic complications or severe infections developed. Out of 229 evaluated patients 126 received 2-CdA with prednisone and 103 received chlorambucil with prednisone. Data obtained from this trial indicate that the OR rate after 2-CdA and prednisone therapy was significantly higher than that after chlorambucil treatment (87% and 57%, respectively, p < 0.001). Moreover, the clinical CR rate after 2-CdA was also significantly higher (47%) than that after chlorambucil treatment (12%) (p < 0.001). Likewise, progression-free survival (PFS) was significantly longer in the 2-CdA treated group (p=0.01) (Figure 2). However, there was no difference in survival duration between the two groups (Figure 3) and no difference in the event-free survival. It should be noted that this trial was designed as a cross-over study and most patients in the chlorambucil group were administered 2-CdA in refractory cases or early relapse. This may have influenced the survival time results.

2-CdA in combination with other agents

Despite the fact that 2-CdA induces higher OR and higher CR rates in patients with CLL, its influence on survival duration is still uncertain and complete recovery is not probable. Combined use

### Table 2. 2-CdA as front line treatment in B-CLL.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of patients</th>
<th>Method of administration</th>
<th>OR (%)</th>
<th>CR (%)</th>
<th>Median duration of response (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saven</td>
<td>20</td>
<td>0.1 mg/kg/d c.i.v. for 7 days, every 28-35 d</td>
<td>85</td>
<td>10</td>
<td>8+</td>
</tr>
<tr>
<td>Juliusson</td>
<td>63</td>
<td>10 mg/m²/d po for 5 days monthly</td>
<td>75</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Tallman</td>
<td>54</td>
<td>0.14 mg/kg/d 2 h i.v. every 28 d</td>
<td>81</td>
<td>26</td>
<td>NA</td>
</tr>
<tr>
<td>Delannoy</td>
<td>19</td>
<td>0.12 mg/kg/d 2 h i.v. monthly</td>
<td>74</td>
<td>47</td>
<td>NA</td>
</tr>
<tr>
<td>Robak</td>
<td>184</td>
<td>0.12 mg/kg/d 2 h i.v. every 28 d</td>
<td>82.5</td>
<td>45</td>
<td>12.0</td>
</tr>
<tr>
<td>Karlsson</td>
<td>61</td>
<td>10 mg/m²/d po for 3 d every 3 weeks</td>
<td>81</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

OR: overall response; CR: complete response; c.i.v.: continuous intravenous infusion; po: orally; i.v.: intravenous infusion; d: day; NA: data not available.

Figure 2. Progression-free survival defined as the time from the end of the first-line therapy to disease progression or death for CLL patients in CR or PR after treatment with 2-CdA+Prednisone (continuous line) and Chl+Prednisone (dotted line). From: Robak T, Blonski JZ, Kasznicki M. Cladribine with prednisone versus chlorambucil with prednisone as first line therapy in chronic lymphocytic leukemia: report of a prospective, randomized, multicenter trial. Blood 2000; 96:2723-9. ©American Society of Hematology, used with permission.

Figure 3. Overall survival time calculated from the first day of treatment to the last day of follow-up or death for CLL patients treated with 2-CdA+Prednisone (continuous line) or Chl+Prednisone (dotted line) as first-line therapy. From: Robak T, Blonski JZ, Kasznicki M. Cladribine with prednisone versus chlorambucil with prednisone as first line therapy in chronic lymphocytic leukemia: report of a prospective, randomized, multicenter trial. Blood 2000; 96:2723-9. ©American Society of Hematology, used with permission.
of 2-CdA with other drugs may increase the CR rate and possibly reduce minimal residual disease (MRD), and prolong survival. Some preclinical in vitro and in vivo studies, as well as early clinical reports, may support such a hypothesis. The reports presenting the results of combined therapy of previously treated and untreated patients with CLL are listed in Table 3.

The combination of corticosteroids with 2-CdA is controversial, because the prolonged immunosuppressive effects of this agent may be enhanced by corticosteroids, resulting in an increased risk of infections, especially opportunistic in nature. We showed that the addition of steroids did not influence the response to 2-CdA and the overall response rates in previously untreated CLL. However, in previously treated patients survival was longer in the group treated with 2-CdA and prednisone than in the group treated with 2-CdA alone. We also investigated the interactions between 2-CdA and dexamethasone on the survival duration of mice bearing lymphoid leukemias L1210 and P388. This study revealed that the combination of 2-CdA with dexamethasone in both leukemias is not more effective than 2-CdA alone. The results of our experimental study are in agreement with clinical observation and indicate that the addition of glucocorticosteroids to 2-CdA does not improve this latter’s antileukemic activity.

Among cytotoxic agents, alkylating drugs were the first candidates for combined use with 2-CdA. Interference of these agents with DNA repair raises the possibility that they might produce synergistic antitumor effects if 2-CdA were combined with cyclophosphamide (CY) or chlorambucil, which acts mainly by cross-linking of DNA strands. In 1993 we demonstrated a synergistic action of 2-CdA and CY on murine leukemia L1210 and P388. Furthermore, Van den Neste et al. in their in vitro study showed that 2-CdA potentiates antileukemic effects of CY derivatives on B-CLL cells. Chlorambucil is another alkylating agent active in CLL for possible combination with 2-CdA. A synergistic antitumor effect of chlorambucil and 2-CdA was seen in CLL in vitro. These agents were also combined in early clinical trials. Tefferi et al. investigated the combination of chlorambucil with 2-CdA in heavily pre-treated patients with CLL. They found that marrow suppression and infections were dose-limiting factors and that the dose of 2-CdA should be lower than when this agent was used alone. Maximaly tolerated doses of 2-CdA combined with standard doses of chlorambucil were also investigated in previously untreated B-cell CLL. Thirty patients received 2 courses of 2-CdA (2 mg/m²/d i.v. for 7 days) with chlorambucil (30 mg/m² orally) given biweekly. The overall remission rate was 80%. The median time to progression in all 30 patients was 30 months. However, similarly to that observed for 2-CdA alone, responses to the combination of the two drugs were not durable and the majority of the patients relapsed within 2 years.

Table 3. Combination of 2-CdA with steroids or other cytotoxic agents in patients with CL.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Previous treatment</th>
<th>No of patients</th>
<th>CR (%)</th>
<th>OR (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-CdAP</td>
<td>-</td>
<td>40</td>
<td>15 (33)</td>
<td>35 (77)</td>
<td>Robak et al.24</td>
</tr>
<tr>
<td>2-CdAP</td>
<td>-</td>
<td>126</td>
<td>59 (47)</td>
<td>102 (87)</td>
<td>Robak et al.1</td>
</tr>
<tr>
<td>2-CdACHN</td>
<td>+</td>
<td>30</td>
<td>6 (20)</td>
<td>24 (80)</td>
<td>Tefferi et al.25</td>
</tr>
<tr>
<td>2-CdACY</td>
<td>+</td>
<td>13</td>
<td>1 (7)</td>
<td>8 (62)</td>
<td>Van den Neste et al.26</td>
</tr>
<tr>
<td>2-CdACHY</td>
<td>+</td>
<td>9</td>
<td>2 (22)</td>
<td>7 (78)</td>
<td>Laurencet et al.27</td>
</tr>
<tr>
<td>2-CdA CY+MIT</td>
<td>+</td>
<td>19</td>
<td>1 (5)</td>
<td>7 (37)</td>
<td>Robak et al.28</td>
</tr>
<tr>
<td>2-CdACHY+MIT</td>
<td>-</td>
<td>62</td>
<td>18 (29)</td>
<td>40 (64.5)</td>
<td>Robak et al.29</td>
</tr>
</tbody>
</table>

2-CdA: 2-chlorodeoxyadenosine, cladribine; P: prednisone; Chl: chlorambucil; CY: cyclophosphamide; MIT: mitoxantrone; CR: complete response; OR: overall response.
Table 4. Updated results of a randomized study comparing the efficacy and toxicity of 2-CdA alone and in combination with cyclophosphamide (CC) or cyclophosphamide and mitoxantrone (CMC) as front-line therapy in B-CLL (number of patients and percentage in parenthesis).

<table>
<thead>
<tr>
<th>Treat.</th>
<th>n</th>
<th>CR</th>
<th>OR</th>
<th>MRD</th>
<th>Grade III or IV Infections Died</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-CdA</td>
<td>67</td>
<td>12</td>
<td>44</td>
<td>9</td>
<td>16 (24) 18 (27) 23 (34) 16 (24)</td>
</tr>
<tr>
<td>CC</td>
<td>77</td>
<td>22</td>
<td>68</td>
<td>6</td>
<td>16 (22) 23 (30) 23 (30) 12 (16)</td>
</tr>
<tr>
<td>CMC</td>
<td>68</td>
<td>22</td>
<td>50</td>
<td>16</td>
<td>18 (26) 32 (47) 36 (53) 11 (16)</td>
</tr>
<tr>
<td>p</td>
<td>0.008 0.005 0.017 0.7</td>
<td>0.028 0.012 0.38</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2-CdA-2-chlorodeoxyadenosine; CC – 2-CdA + cyclophosphamide; CMC – 2-CdA + mitoxantrone + cyclophosphamide; CR – complete response; OR- overall response; MRD – minimal residual disease evaluated by flow cytometry in the patients with CR.

Chronic lymphocytic leukemia: is it a curable disease?

The combination of these antibodies with 2-CdA may increase the CR rate, decrease MRD and prolong survival. However, so far there have been no clinical studies to support this hypothesis.

Early and late complications of 2-CdA treatment

Bone marrow suppression with anemia, neutropenia and thrombocytopenia is the dose-limiting factor for 2-CdA. Prolonged thrombocytopenia, neutropenia and anemia were observed particularly after multiple courses of therapy and in heavily pre-treated patients. A randomized study confirmed a strong myelosuppressive effect of 2-CdA, which resulted in a higher incidence of neutropenia and infections in patients treated with 2-CdA and prednisone than in patients treated with chlorambucil and prednisone. Moreover, treatment with 2-CdA leads to a decrease in the CD4+/CD8+ ratio for an extensive period of time, exceeding even 24 months. In consequence, infections, including opportunistic ones, are frequent events and infections with fatal outcome have been reported.

Some reports suggest that 2-CdA may induce autoimmune hemolytic anemia (AIHA) in patients with CLL despite the reduction in the leukemic clone. However, the results of a randomized study did not support this hypothesis. In our randomized study AIHA was noted in 7 patients treated with 2-CdA and in 2 patients treated with chlorambucil, but this difference was not statistically significant (p=0.3).

Pure red cell aplasia (PRCA) occurs in approximately 5% of CLL patients, most often later in the course of disease. Some reports suggest that treatment with nucleoside analogs may induce PRCA. We reviewed the records of 470 patients with B-CLL treated with 2-CdA alone or in combination and analyzed the occurrence of PRCA, as well as the effect of 2-CdA in patients with co-existence of these diseases. PRCA was diagnosed in 8 patients, including 3 with PRCA noticed before the initiation of 2-CdA, and 5 with PRCA diagnosed shortly after treatment with nucleoside analogs. The frequency of PRCA seems to be higher in patients treated with 2-CdA in combination with cyclophosphamide or cyclophosphamide and mitoxantrone (4.3%) than in those treated with 2-CdA alone (0.5%). If 2-CdA was started after the diagnosis of PRCA, no improvement of erythroid parameters was observed. Taken together, our retrospective analysis showed that PRCA is not more frequent in B-CLL patients treated with 2-CdA.
alone than in other patients with CLL described in the literature. However, the frequency of PRCA may increase when 2-CdA is combined with other cytotoxic agents.

A rare complication of 2-CdA treatment in patients with CLL is tumor lysis syndrome. Dann et al. described the occurrence of this complication after the infusion of 2-CdA in a patient with CLL and bulky lymphadenopathy.44 The patient died after 10 days of hospitalization. Prolonged immunosuppression related to 2-CdA treatment may increase the risk of secondary malignancies in patients with CLL. Some authors observed secondary MDS/AML in patients treated with this agent.45,46 In our randomized study comparing 2-CdA and prednisone with chlorambucil and prednisone we observed secondary malignancies only in two patients treated with 2-CdA and in one patient treated with chlorambucil.7 However, longer observations are needed to solve this problem.

Another question is the influence of 2-CdA treatment on the development of Richter’s syndrome (RS). In a randomized study RS was observed in 3 (2.4%) patients treated initially with 2-CdA and in 4 (3.9%) patients treated with chlorambucil.7 More recently, we have diagnosed two unusual cases of RS in CLL patients treated with 2-CdA.47,48 In one patient isolated diffuse large B-cell lymphoma (DLBL) developed in the femur one year after completion of the 6th course of 2-CdA.47 In the second patient DLBL of the plasmablastic type developed 1.5 years after the 10th course of 2-CdA treatment.48 However, the estimation of the real incidence of RS in CLL patients treated with 2-CdA needs further observation and longer follow-up.

Is 2-CdA equivalent to fludarabine in B-CLL?

2-CdA is structurally similar to FAMP. Unfortunately, results of a randomized study comparing the activity of both agents in CLL have not been published so far. Moreover, there is less experience with the use of 2-CdA than FAMP in patients with CLL in the majority of Western countries. However, similarly to FAMP, this agent has been found to be more effective in previously untreated CLL patients than in patients refractory to, or relapsing after conventional therapy.6 In refractory or relapsed patients 2-CdA induces a CR rate of 0-31% and an overall response rate of 31-68%. In previously untreated patients the overall response rate is 74-85% and CR 10-47%. These results are similar to those obtained with FAMP. This opinion has been also indirectly supported by in vitro and in vivo studies. Begleiter et al. compared in vitro anti-tumor activity of these two agents in leukemic cells from 28 patients with CLL.49 They showed that cells from 90% of the studied patients had similar relative orders of sensitivities to 2-CdA and FAMP. In contrast, only 10% showed different sensitivity. Similar results were obtained by Koski et al., who determined the chemosensitivity of leukemic cells obtained from the peripheral blood of 35 patients with B-CLL by a leucine-incorporation assay.49 They found a good correlation between the sensitivity to 2-CdA and FAMP among previously untreated patients when tested at the 80% inhibition level.

Finally, one phase II randomized study performed in previously treated low grade lymphoma patients showed that 2-CdA and FAMP gave similar response rates and duration.50 It should be noted that an international randomized study comparing 2-CdA, FAMP and chlorambucil in previously untreated patients with CLL has been initiated by Juliusson et al. The results from this study should definitely prove the similar value of 2-CdA and FAMP in CLL patients.

Funding

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References

Chronic lymphocytic leukemia: is it a curable disease?


18. Robak T, Blonski JZ, Kasznicki M. Does intensive treatment with high dose chlorambucil and prednisone as first line and cladribine as second line influence the survival of the patients with chronic lymphocytic leukemia? Leuk Lymphoma 2001; 41:545-57.


36. Chow KU, Rummel MJ, Weidmann E, Ries J, Jantschke P, Boehrler S, et al. Induction of apoptosis by 2-chloro-2'-deoxyadenosine (2-CdA) alone and in combination with other cytotoxic drugs: synergistic effects on normal and neoplastic lymphocytes by addition of doxorubicin and...


MabCampath is clearly an extremely effective therapy in refractory chronic lymphocytic leukemia (CLL). It is probably the single agent most effective at lysing CLL cells but is intensely immunosuppressive and therefore creates novel problems of supportive care. That MabCampath has a role in the therapy of CLL is undeniable but the best place for this drug in the therapy of CLL remains to be determined.

MabCampath is a humanized monoclonal antibody directed against CD52, an antigen expressed on all lymphocytes. In studies of MabCampath for the treatment of autoimmune conditions, in particular rheumatoid arthritis, profound and protracted lymphopenia was observed. The remarkable efficiency with which MabCampath depletes lymphocytes led to its investigation as an agent for the treatment of lymphoproliferative disorders, such as chronic lymphocytic leukemia. Phase I and II studies in a variety of lymphoproliferative disorders (LPD) were performed in the late 1980s and early 1990s. The two major conclusions drawn from these studies were: 1) that MabCampath appeared more effective in chronic lymphocytic leukemia than in the LPD with predominantly nodal disease; and 2) the best tolerated effective intravenous dosing schedule was 30 mg three times a week.

Two phase II studies (125-005 and 125-009) of MabCampath utilizing the above dosing regime in patients with CLL who were refractory to conventional therapies were performed. A total of 64 patients were enrolled onto these studies: all had failed prior therapies and the majority had previously received purine analogs. The overall response rate according to NCI criteria was 27%. Prophylactic antibiotics were not routinely used and a low but significant incidence of Pneumocystis carinii pneumonia (PCP) was observed. The results of these studies were submitted to the regulatory authorities (FDA and EMEA) in 1996 and a further study was requested in a more uniform group of refractory patients: this study was called the CAM211 study.

The patients in CAM211 had all failed fludarabine therapy by strict criteria (either no response or relapse within 6 months of completing treatment) and had previously received alkylating agents. Based on historical controls, the expected median survival of this group was approximately 9 months. Since there was no therapy licensed for such patients and no consensus on the most appropriate alternative therapy a randomized, controlled trial was considered difficult to defend ethically. It was, therefore, agreed that an objective response rate of 20% maintained for at least 6 months, when assessed by an independent panel, would constitute sufficient evidence of the efficacy of MabCampath in this clinical setting. In the CAM211 study, following the experience of the previous studies, patients received prophylaxis against PCP with co-trimoxazole as well as against Herpes reactivation with famciclovir (or acyclovir). No cases of PCP were observed in patients on prophylaxis. A total of 93 patients were enrolled into CAM211 in both Europe and USA. The overall response rate in CAM211 was 33% with 29 partial remissions and 2 complete remissions. In addition, approximately 70% of patients with anemia, neutropenia and/or thrombocytopenia prior to MabCampath had a significant improvement in marrow function and 27/38 (71%) of patients had a complete resolution of their B-symptoms. The median survival of the patients entered into CAM211 exceeded that expected at 16 months. A better response rate was observed in patients without massive lymphadenopathy. Cytomegalovirus (CMV) reactivation occurred in 6% of patients in the pivotal studies (125-005, 125-009 and CAM211) and although most cases were considered severe...
(grade 3 or 4) all were managed successfully with appropriate anti-viral therapy. These results were submitted to the regulatory agencies (FDA and EMEA) who considered that MabCampath was proven to be an effective agent in the treatment of chemotherapy-refractory CLL and issued a Product Licence in 2001. It is clear that MabCampath is a therapy with a unique side effect profile that can be safely managed with appropriate prophylaxis against and monitoring for opportunistic infections. The selection of patients is critical to achieve the best response and to limit side effects.

The administration of MabCampath is not without its difficulties. The routine dosing regime of 30mg by a 2-hour infusion three times a week for up to 12 weeks is extremely inconvenient for nursing/medical staff and patients. The infusion-related reactions, particularly fever and rigors that occur in most patients, can be very difficult to control and extremely distressing for patients. The occurrence of other side effects, such as the transitory neutropenia seen in the first few weeks of many patients’ therapy, the potential for opportunistic infections, particularly cytomegalovirus reactivation and the risk of PCP, are potential pitfalls. Also, as can be seen from the evidence from the pivotal studies, although in this difficult group of refractory patients the response rates are quite impressive, the majority of patients do not respond and non-responders have a dismal survival. Thus approaches which may optimize responses to MabCampath and minimize its side effects are currently being explored.

Management of complication
Infusion-related complications
Infusion-related complications can usually be managed successfully with paracetamol and antihistamines prior to the infusion. Some patients may require steroids to prevent reactions but it is prudent to keep the use of corticosteroids to a minimum in view of the theoretical risk of increasing the incidence of opportunistic infections when steroids are combined with MabCampath. Another approach to minimizing infusion-related problems caused by MabCampath is to administer the drug via the subcutaneous route. This route is associated with far less severe systemic reactions but is associated with local injection site reactions that can be severe. It needs to be established that the subcutaneous route is as effective as the intravenous route.

Prevention and management of opportunistic infections
Prophylaxis against PCP is mandatory in view of the small but definite risk of PCP infection. CMV infection is perhaps the most feared opportunistic infection occurring during MabCampath therapy and once a patient has symptoms of CMV his or her prognosis is poor. A more effective strategy is to monitor weekly for CMV reactivation by polymerase chain reaction (or antigen testing) and to treat with ganciclovir pre-emptively when screening becomes positive. In this way CMV infection is usually associated with relatively mild or no symptoms.

Management of cytopenias
MabCampath therapy is frequently associated with neutropenia and, to a lesser extent, thrombocytopenia. The cause for these cytopenias is unclear – CD52 is not expressed on and is not directly toxic to hematopoietic stem cells. One possible explanation is that as the CLL in the marrow lyses then the normal hematopoietic progenitors are inhibited. In support of this theory is the observation that the cytopenias recover whilst the patient remains on MabCampath suggesting that the infiltration with CLL is improving and hence bone marrow function recovers. Often the neutrophil count drops to a half or even a third of the level at the start of therapy. The most effective management is to prevent severe neutropenia by using granulocyte colony-stimulating factor (G-CSF). G-CSF given on the days of MabCampath (3 times a week) to maintain the neutrophil count above 1.0×10^9/L is effective. Most patients treated in this way will respond to G-CSF thus avoiding unnecessary delays in MabCampath therapy. Thrombocytopenia is less of a problem and usually requires no intervention.

Optimizing responses to MabCampath
Monitoring responses. The rapid depletion of peripheral blood lymphocytosis is a remarkable feature of MabCampath therapy. The peripheral blood lymphocyte count is usually undetectable by the end of the second week of therapy. Despite this rapid clearance of the peripheral blood, the clearance of CLL from the bone marrow is far less rapid. After 4 weeks therapy the bone marrow is very rarely significantly improved: it is not until week 8 that the marrow starts to improve and week 12 that the marrow has usually responded maximally. Therefore monitoring the peripheral blood is of little use in assessing response to MabCampath therapy. In patients who do not have significant
lymphadenopathy it is essential to perform bone marrow examinations in order to assess the response and to define the duration of response. There are compelling reports suggesting that if detectable CLL can be eradicated from the bone marrow, then survival is prolonged. Thus it is optimal to assess the marrows on each occasion with sensitive techniques, such as multi-parameter flow cytometry.

Optimizing responses to MabCampath. Combination therapy: approximately one third of patients receiving MabCampath for refractory CLL will achieve an adequate response. Effective therapeutic avenues for the remaining patients are extremely limited and therefore optimizing the responses to MabCampath is essential. In view of the fact that MabCampath appears to be most effective for disease in the blood and marrow whereas rituximab, at least in follicular lymphoma, appears to act preferentially on nodal disease there has been some work combining the two antibodies (MabCampath + rituximab). Alternatively, MabCampath combined with fludarabine appears to be effective.

In an initial report on 6 patients, five had a significantly better response to the combination than to either drug alone and 3 of the 6 obtained morphologically normal bone marrow.

MabCampath earlier in the disease. Anders Osterborg’s group from the Karolinska Institute in Sweden have recently reported the use of subcutaneous MabCampath in front-line patients with CLL. They treated 41 previously untreated patients with CLL with 30mg MabCampath subcutaneously three times a week for up to 18 weeks, depending on response. The response rate of 87% (CR [29%] + PR [68%]) is comparable to that obtained with other first-line therapies with an acceptable toxicity.

There are on-going studies with MabCampath as consolidation therapy for patients with a complete or partial response to conventional therapies. The key factor which will dictate whether MabCampath has a role earlier in CLL is whether the inevitable complications linked to immunosuppression are outweighed by the benefits of better remissions. This fundamental question is some way from being answered.
The first publications examining the use of monoclonal antibodies (MAb) in the treatment of lymphoid malignancies were published in 1980. However, interest in using MAb waned, due in part to the initial problems encountered. The first issue with any MAb is the specificity of the antigen. Because there is no true tumor antigen for virtually any of the neoplastic diseases, it is necessary to target cell-surface antigens which are only partially specific. Unfortunately, this means that there will always be overlap, and normal cells will also be targeted.

All of the original MAb were murine. One problem with murine MAb was the inability to shrink tumors, presumably because murine antibodies did not bind well to human effector cells or complement. Additionally, the window of opportunity for using murine MAb was limited due to the fact that patients rapidly formed antibodies against the murine MAb (human antimouse antibodies [HAMA]), which resulted in rapid clearing of the murine MAb.

Chimerization is one of the main advances in MAb technology and has led to the development of currently available MAb. A humanized antibody is one that retains the murine portion of the variable region that binds to the antigen, but the Fc fragment of the antibody is a human sequence. Humanization of MAb has 2 major advantages:

- humanized MAb can bind human effector cells;
- patients will not develop antibodies to the humanized MAb antibody, which will enable the patient to receive MAb therapy repeatedly.

Rituximab

Rituximab is a MAb targeted against the CD20 transmembrane antigen on malignant and normal B-cells. There was interest in using rituximab to treat patients with chronic lymphocytic leukemia (CLL) based on results from the low-grade lymphoma trial, in which the standard dose of 375 mg/m² weekly for 4 weeks produced an overall response rate of approximately 50%. Notably, there was a striking disparity in the response rate according to histology. Patients with follicular lymphoma had a 60% response rate whereas patients with well-differentiated lymphocytic lymphoma or small lymphocytic lymphoma had a response rate of only 12%.

Patients with well-differentiated lymphocytic lymphoma or small lymphocytic lymphoma, as well as patients with CLL, have less CD20 expression on the surface of their cells than do patients with follicular lymphoma. Also of interest was the fact that pharmacokinetic studies showed that those patients had very rapid clearance of rituximab.

At the University of Texas M.D. Anderson Cancer Center, an assay to measure CD20 that is no longer attached to the B-cell surface (soluble CD20) has been developed. Using this assay system, Albitar et al. showed that there are detectable levels of soluble CD20 in the serum of essentially all patients with CLL. One possibility is that the soluble CD20 could explain the more rapid clearance of the rituximab antibody and thus the lower response rates.

Based on these data there was concern about performing a phase two trial in patients with CLL using the standard dose of rituximab and achieving a response rate of only 10-15%. Thus, a dose escalation study was performed, and conducted in the manner of a phase 1 trial. Acknowledging that because rituximab is a MAb, and not chemotherapy, a true maximum tolerated dose (MTD) might not be determinable it was decided that if sustained toxicity were observed with a specific dose of rituximab, the escalation would be terminated. There was significant concern that administering the antibody to patients with high circulating tumor burden could result in more severe initial side effects. In the interest of safety, a dose of 375 mg/m² was given as the first dose to all patients.
Subsequently the dose was escalated, although held constant in an individual patient, over the next 3 weeks for a total of 4 weeks of therapy. The highest dose achieved in this trial was 2,250 mg/m². A true MTD was not determined.

Fifty patients (40 patients with CLL, 4 patients with marginal zone leukemia, 2 patients with prolymphocytic leukemia, and 4 patients with mantle cell leukemia) participated in the study. All of the patients, except 1 patient with marginal zone leukemia, had been previously treated. Approximately 50% of the patients were refractory to fludarabine.

Ninety-four percent of the patients exhibited some form of toxicity with the first dose of rituximab, predominantly fever and chills of grade 1-2 toxicity. Six patients (12%) had severe toxicity with the first dose of the antibody, including high fever, chills, dyspnea, hypoxia in all, and 5 patients had hypotension. Among the 40 patients with CLL, there was only 1 severe reaction, and among the 10 patients with other B-cell leukemias, there were 5 severe reactions. Notably, the cell surface antigen expression of CD20 is much greater in these other B-cell leukemias than in CLL. These findings suggest that infusion-related reactions to rituximab may not be related to the height of the white blood cell count, but may depend on the amount of cell surface CD20. Given circulating cells that intensely express CD20, it is likely that there will be a brisk lysis of cells and release of cytokines. Two of the cytokines that are released quickly are tumor necrosis factor-α and interleukin-6, which may contribute to the side effects that are observed with rituximab treatment.

Evaluation of the toxicity during the rituximab dose escalation indicated that up to a dose of 1,500 mg/m², minimal side effects were observed. In the few cases in which toxicity was seen, it was grade 1. At a dose of 2,250 mg/m², 8 of the 12 patients exhibited moderate toxicity, and dose escalation was terminated. No grade 3 to grade 4 toxicity was seen.

The overall response rate was 40%; all of the responses were partial remissions except for 1 complete remission which was observed in a patient with prolymphocytic leukemia. Unlike what was noted in the Campath-1H studies, no significant difference in response rate based on lymph node size was observed. Patients who had fludarabine refractory disease had a significantly lower response rate. There were 7 evaluable patients with other B-cell leukemias, 6 of whom responded. While these numbers were small, it seems that there may be an important role for this antibody in the treatment of other mature B-cell leukemias.

Several conclusions were made from this trial:

- Rituximab showed significant activity in patients with CLL;
- there appeared to be a dose response;
- the toxicity profile was acceptable.

Byrd and colleagues also evaluated a dose intensive schedule of rituximab in CLL. They administered the standard dose of 375mg/m² but gave this agent three times weekly for four weeks. Thirty-three patients were treated, 26 with CLL and 7 with SLL. The median number of prior regimens was 2; 6 patients had received no prior chemotherapy. The overall response rate in this trial was 45% and there was no difference in response based on diagnosis (CLL vs SLL), Rai stage or lymph node size. Five of the 6 patients who were previously untreated responded (83%). No complete responses were noted.

The use of rituximab as initial therapy for CLL has been described by two groups; Thomas et al. gave rituximab 375 mg/m² IV weekly for eight weeks to patients with Rai stage 0-2 CLL who did not have indications for therapy using standard

**Table 1.** Rituximab for CLL: standard schedule.

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>CR (%)</th>
<th>OR (%)</th>
</tr>
</thead>
<tbody>
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<td>—</td>
<td>10</td>
</tr>
<tr>
<td>Winkler, 1999</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Huhn, 2001</td>
<td>28</td>
<td>—</td>
<td>25</td>
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</tbody>
</table>

**Table 2.** Rituximab for CLL: effect of increased dose/dosing frequency.

<table>
<thead>
<tr>
<th>Study</th>
<th>Regimen</th>
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<td>3</td>
<td>45</td>
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<tr>
<td>O’Brien, 2001</td>
<td>Escalation</td>
<td>40</td>
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<td>Mid (1,000-1,500 mg/m²)</td>
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<td></td>
<td>High (2,250 mg/m²)</td>
<td>75</td>
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NCI working group criteria, but were considered at high risk for progression based on a β2 microglobulin level > 2.0 mg/L. Thirty patients were treated in this pilot trial and the overall response rate was 83% with a 17% complete response rate. Hainsworth et al. also administered rituximab to previously untreated patients with CLL but these patients had advanced stage disease. Seventy patients were treated, 39 with SLL and 31 with CLL. They received rituximab at 375 mg/m² weekly × 4 and additional consolidation treatments of four weekly doses of rituximab were given every six months for four cycles. Fifty-six patients were evaluable and the overall response rate was 44% with 9% complete response. Follow-up was limited but the estimated median progression-free survival was thirty-five months.

Another interesting approach is to combine monoclonal antibodies for the treatment of CLL. Under the direction of Dr. Stefan Faderl we have been conducting a trial evaluating Campath-1H and rituximab for the treatment of CLL. The rationale for this approach is that these agents bind to different surface antigens and may trigger different downstream pathways that can result in apoptosis. Both are clinically active agents in the treatment of CLL and they are complementary in that their sites of major efficacy are different, with Campath-1H preferentially targeting the bone marrow and showing less efficacy in bulky lymph node sites whereas single agent rituximab effectively reduces lymph node size in CLL but has little effect on marrow disease. The eligibility for this trial included any refractory acute/chronic lymphoid malignancy in which the cells expressed both CD52 and CD20. Previous exposure to either single agent was allowed.

Rituximab was given at a dose of 375 mg/m² on day one weekly for four weeks (i.e. standard dosing of rituximab). In the first week Campath was given with a stepped up dosing regimen of 3 mg, 10 mg, 30 mg on three consecutive days and then subsequently given at 30mg twice a week (2/3 of the standard weekly dose for the single agent). Twenty-five patients have been treated, the majority of them with CLL or CLL/PLL. The median age was 60 years (range 49-79). The median number of prior regimens was 4 (range 1-8). Seventy-two percent of the patients had advanced stage disease and 54% were refractory to fludarabine. The overall response rate thus far is 44%. Eight of fifteen patients with CLL (53%) responded, as did one of five patients with CLL/PLL. A single patient with marginal zone leukemia responded and one patient each with mantle-cell leukemia, prolymphocytic leukemia and two patients with Richter’s transformation have not responded. Toxicities were predominantly infusion-related side effects seen with the initial dosing of both agents. Grade 3-4 infusion-related toxicities were rare. Infections have been seen in thirteen of the twenty-five patients including four with fever and CMV antigenemia. These toxicities are no different from those that would be expected from the use of either single agent. The median time to progression has not been reached with a short median follow-up of about six months. The efficacy of the combination appears promising, particularly as the duration of therapy combining both agents is brief, being one month.

Future directions for use of MAb treatment have included combining chemotherapy and MAb therapy to exploit a potential synergy in the treatment of CLL.

A regimen was designed at the M.D. Anderson Cancer Center in which all patients received the standard dose of rituximab (375 mg/m²) on day 1, followed by 3 days of chemotherapy (25 mg/m² of fludarabine and 250 mg/m² of cyclophosphamide) on days 2 through 4. In the second and subsequent
Chronic lymphocytic leukemia: is it a curable disease?

Several conclusions can be made concerning the use of rituximab in the treatment of CLL. The use of the single agent at standard doses produces a low response rate but a dose intensified regimen achieved by giving more frequent doses or by escalating the dose appears to produce higher response rates. The use of rituximab in combination with chemotherapy appears to potentiate this chemotherapy particularly in terms of increasing the CR rate and molecular remissions can also be achieved. Neutropenia is more common but thus far this has not resulted in significantly greater infection rates. The ability to achieve molecular remissions would suggest that there will also be an impact on remission duration although the follow-up in the current trials is too early to provide these data.

References


Abnormalities in the cell cycle are responsible for the majority of human neoplasias. The key regulators of the cell cycle are the cyclin-dependent kinases (CDKs), enzymes that periodically form complexes with proteins known as cyclins. Mutations and/or deletions in some cell cycle proteins can result in the inactivation of the retinoblastoma (Rb) gene product. Such mutations are responsible for the development of human neoplasia. Therefore, a pharmacologic cyclin-dependent kinase inhibitor is of great theoretical interest as a treatment strategy for many neoplasms. Flavopiridol (NSC 649890, HMR 1275) is a flavonoid with potent CDK inhibitory activity. Moreover, flavopiridol has the capacity to decrease the expression of cyclin D1 and vascular endothelial growth factor (VEGF) mRNA by transcriptional and post-transcriptional mechanisms. Recently we demonstrated that flavopiridol inhibits positive elongation factor B (P-TEFb), a complex composed of CDK9 and cyclin T, leading to a block in HIV replication. In preclinical models of lymphoid and head and neck cancers, flavopiridol induced apoptosis irrespective of the presence of BCL-2 or p53. The first phase 1 trial of a CDK inhibitor, flavopiridol, has been completed. Main side effects noted were secretory diarrhea and pro-inflammatory syndrome. Antitumor activity was observed in patients with non-Hodgkin’s lymphoma and renal, colon, and prostate cancers. Concentrations between 300 and 500 nM—necessary to inhibit CDK and achieve an antiproliferative effect—were reached safely. Phase 2 trials with infusional flavopiridol and phase I infusional trials in combination with standard chemotherapeutic agents including cisplatin, paclitaxel, docetaxel, etoposide and carboplatin are being completed with encouraging results. We have recently completed a novel phase I trial of 1 hour flavopiridol administration. The maximum tolerated dose using flavopiridol daily for 5, 3 and 1 consecutive days are 37.5, 50 and 62.5 mg/m²/day. Dose-limiting toxicities include vomiting, neutropenia, pro-inflammatory syndrome and diarrhea. Plasma flavopiridol concentrations achieved were ~1.5-3.5 µM. Phase II trials using this schedule in several tumors including chronic lymphocytic leukemia, mantle cell lymphoma, head and neck and lung cancer, among others, are being conducted worldwide.

UCN-01 (7-hydroxystaurosporine; NSC 638850), the second CDK modulator that has entered into clinical trials, can inhibit CDK activity at concentrations 10 times higher than those required to inhibit protein kinase C; moreover, UCN-01 can modulate CDK activity at much lower concentrations by affecting the phosphorylations that regulate such activity. UCN-01 showed potent apoptotic and cell cycle effects in different in vitro models. Moreover, UCN-01 abrogates the G2 arrest induced by DNA-damaging agents in cells lacking normal p53 function due to modulation of CDK1 activity. These results could suggest a novel strategy to combine UCN-01 with DNA-damaging agents. In the initial clinical trial, in which UCN-01 was administered by continuous infusion for 72 hours, a prolonged half-life of about 600 hours (roughly 100 times longer than the half-life seen in preclinical models) was observed. The maximum tolerated dose was 42.5 mg/m²/day. Dose-limiting toxicities were nausea/vomiting, hypoxemia and symptomatic hyperglycemia. One patient with refractory melanoma achieved a partial response (8 months). Another patient with refractory anaplastic large cell lymphoma is NED for more than 4 years. In an effort to determine the pharmacodynamic effects of UCN-01, we determined prospectively, from tumor and bone marrow samples, the activity of protein kinase C (PKC) as measured by Western blot analysis against phosphorylated α-adducin, a substrate of PKC. Loss of phospho-adducin was observed. Phase I trials with shorter infusions are being completed. In summary, the
first two CDK modulators have shown encouraging results in early clinical trials. Questions that remain unanswered are: what is the best schedule for administering these agents, which standard antitumor agents are the best for combination strategies with these CDK modulators, and which pharmacodynamic endpoint reflects loss of CDK activity in tissue samples from patients in these trials? Despite these caveats, we feel that CDK are sensible targets for cancer therapy and, that several small molecule CDK modulators are showing encouraging results in clinical trials.

References

The Role of:

New drugs: can they cure chronic lymphocytic leukemia?

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research paper

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N
ovel strategies are needed to improve the prognosis of patients with chronic lymphocytic leukemia (CLL). Fludarabine has been shown to be the most effective agent for this disease, but does not produce a clear prolongation of survival. Therefore, newer and more active agents are needed.

Depsipeptide

Depsipeptide (NSC 630176) is a bicyclic peptide originally isolated from Chromobacterium violaceum, strain 968, by Fujisawa Pharmaceutical Co., Ltd. Depsipeptide inhibits tumor cells through histone acetylation. Other investigations on the effect of depsipeptide on G1 to S transition of the cell cycle showed that depsipeptide inhibits signal transduction through MAP kinase and causes p53-independent G1 arrest. Depsipeptide, either alone or in combination with hypomethylating agents, has been shown to induce a number of cellular proteins that may have critical effects on apoptosis, proliferation and susceptibility to immunologic manipulation. The compound may also have an antiangiogenic activity that contributes to anti-tumor efficacy. Depsipeptide also decreases the viability of CLL cells. Depsipeptide was more cytotoxic to B-PLL cells, when compared to F-ara-A, gemcitabine, flavopiridol, and UCN-01, and had much more pronounced activity (1.6×10^3-fold greater) against B-PLL cells than normal peripheral blood mononuclear cells.

In phase I and II trials in patients with peripheral T-cell non-Hodgkin's lymphoma (NHL) or mycosis fungoides conducted at the NCI, objective responses were reported in 73% of evaluable patients, including two complete responses (CR) (S. Bates, personal communication). Toxicities attributed to depsipeptide included anemia, leukopenia, neutropenia, thrombocytopenia, fatigue, anorexia, nausea, vomiting, elevated AST/ALT, increased CPK, hypocalcemia, asymptomatic EKG changes (ST-T wave flattening and inverted T waves), and supraventricular arrhythmias (SVT/atrical fibrillation/flutter).

PS-341

The proteasome is a large, multicentric protease complex with a pivotal role in cellular protein regulation. The proteasome degrades proteins that have been conjugated to ubiquitin, resulting in what is referred to as the ubiquitin-proteasome pathway. The ubiquitin-proteasome pathway plays a critical role in the degradation of intracellular proteins involved in cell cycle control and tumor growth. The proteasome is also required for activation of NFκB which plays a role in maintaining cell viability through the transcription of inhibitors of apoptosis. Since NFκB can induce drug resistance, this agent may make cells more chemosensitive.

PS-341, a dipeptidyl boronic acid, is a specific and selective inhibitor of the 26S proteasome. It plays a regulatory role in multiple cellular pathways involving cell cycle, transcription factor activation, and cell trafficking. PS-341 may also induce apoptosis. PS-341 has shown activity against cell types characterized by overexpression of BCL-2. Increasing evidence supports the use of proteasome inhibitors in the therapy of patients with CLL. First, the ubiquitin-proteasome-dependent protein processing may be altered in CLL cells. In addition, proteasome inhibition has been shown to induce apoptosis of CLL lymphocytes at concentrations which do not have that effect on normal cells. Activity against CLL and mantle cell NHL has been observed in phase I studies, and more than 50% of patients with refractory multiple myeloma respond to this agent.
Antisense oligonucleotides

Antisense oligonucleotides are chemically modified single-strand DNA molecules that have a nucleotide sequence that is complementary to the target mRNA and are, therefore, capable of inhibiting expression of that target gene. The Bcl-2 gene is a potentially important target because it is over-expressed in most follicular B-cell non-Hodgkin's lymphomas and chronic lymphocytic leukemias and in about a quarter of large B-cell NHL. Bcl-2 upregulation is thought to be responsible for maintaining the viability of tumor cells as well as inducing a form of multi-drug resistance. Elevated Bcl-2 levels also correlate with poor response to therapy in NHL, AM and possibly CLL. These observations, and others, have stimulated interest in exploring an antisense oligonucleotide against Bcl-2 and other genes important to tumor survival.

Antisense oligonucleotides must first be incorporated into cells by endocytosis in order to be effective. The oligonucleotide then inhibits gene expression by hybridization with the mRNA, followed by cleavage of the mRNA by recruitment of RNase H. Critical to the function of these oligonucleotides is their resistance to nuclease digestion.

G3139 (oblimersen sodium; Genta Incorporated, Berkeley Heights, NJ, USA), the first antisense molecule to be widely tested in the clinic for the treatment of human tumors, is a phosphorothioate oligonucleotide consisting of 18 modified DNA bases (i.e., 18-mer) that targets the first 6 codons of Bcl-2 mRNA to form a DNA/RNA duplex.

In vitro studies showed synergistic enhancement of tumor cell killing when the Bcl-2 antisense oligonucleotide was used to reduce Bcl-2 protein content in combination with antimetabolites, alkylators, corticosteroids, other cytotoxic chemotherapy, radiation, and monoclonal antibodies. B-cell lines demonstrated significant enhancement of cytotoxicity after reduction of Bcl-2 produced by antisense treatment. In addition, fresh CLL cells obtained from patients and studied ex vivo have been shown to demonstrate downregulation of Bcl-2 protein by antisense exposure, leading also to increased susceptibility to killing by fludarabine.

In the first phase I study of G3139 in patients with NHL 13, dose-limiting toxicities included thrombocytopenia, hypotension, fever, and asthenia. Despite the low doses of drug used in this trial, Bcl-2 downregulation was achieved, and several major responses were observed.

Phase I-II trials in other malignant disorders indicate that the most common toxicity associated with the agent is fatigue. Liver function abnormalities and thrombocytopenia have been observed but are usually transient and have not delayed drug dosing.

In a phase I-II study of 14 patients with CLL, doses of G3139, at 4, 5 or 7 mg/kg/day for 5–7 days were poorly tolerated with fever, hypotension, back pain, and thrombocytopenia. Nevertheless, antitumor activity was indicated by an episode of tumor lysis in one patient, a reduction in lymphadenopathy in 4 patients, a decrease in splenomegaly in 1 patient, and a transient decrease in circulating lymphocytes of at least 33% in 5 patients. This phase I trial established that the maximum tolerated dose for cycle 1 in CLL as monotherapy is 3 mg/kg/day, although the dose could be safely escalated to 4 mg/kg/day in subsequent cycles. An ongoing phase III trial is comparing fludarabine plus cyclophosphamide with or without G3139 in patients in whom prior fludarabine therapy has failed.

In vitro study of NHL cell lines showing marked synergy between G3139 and rituximab has led to clinical trials in CLL and NHL evaluating the combination of these two agents.

Promising new chemotherapy agents with unique mechanisms of action are currently in clinical trials. Future strategies must be directed at attacking appropriate therapeutic targets using rational combinations of these drugs and other new compounds. It is important to accrue patients quickly to clinical trials so that effective new approaches with the goal of curing patients with CLL are rapidly made available.

References

Chronic lymphocytic leukemia (CLL) has a highly heterogeneous clinical course, with indolent forms requiring no treatment and more aggressive varieties showing a survival likelihood <3 years when conventionally treated. Though the overall median age at diagnosis is about 65 years, more than 30% of CLL patients are younger than 60 years at the time of presentation.1-3 Patients' age is very important for designing therapy especially when, in the presence of adverse prognostic factors, intensive treatment including high-dose therapy followed by hematopoietic precursor cells are envisaged in an attempt to provide long-lasting remissions and possibly to eradicate the disease. Recently, the use of autografting procedures for patients with CLL has increased worldwide particularly because (a) collection and utilization of sufficient numbers of hematopoietic precursor cells from the blood has improved; (b) better quality remissions after fludarabine therapy are achieved; and (c) appropriate management of the complications to which CLL patients are prone has ameliorated. A growing number of published single center series and retrospective analyses from the EBMT and IBMTR groups have reported an overall survival between 50-80% after transplantation with a transplant-related mortality ranging from 4 to 19%. A better outcome had been reported in patients with sensitive disease transplanted in complete remission (CR) and in an early stage of the disease, while a beneficial effect of several methods of purging has not been demonstrated.4-11

In Italy, two pilot studies were recently conducted in advanced stage CLL patients. One was carried out by the Palermo group. The data on the collection and manipulation of progenitors demonstrated the feasibility of a mobilization, purification and autografting program with peripheral blood stem cells (PBSC) in patients responding to initial treatment with fludarabine.

In this limited series, 11 and 16 days were necessary for the recovery of $0.5 \times 10^9/L$ granulocytes and $25 \times 10^9/L$ platelets, respectively. At a median follow-up of 16 months, 9 patients are alive and 8 in continuous CR.12 The second study was conducted in Rome, at the Biotecnologie Cellulari ed Ematologia Unit of La Sapienza University. Twenty patients were autografted following fludarabine treatment using BEAM as conditioning regimen. In no case were purging methods applied.

All patients engrafted, with a median time to the recovery of $>20 \times 10^9/L$ platelets and $>0.5 \times 10^9/L$ neutrophils of 15 and 12 days, respectively. The probability of survival at 52 months from transplant is 87%.13

These preliminary results represented the basis for the design of a randomized study of autologous transplantation vs. fludarabine alone in CLL. The program also includes a series of biological and molecular studies addressed at evaluating minimal residual disease during the course of treatment.

Design and Methods
The primary objective of the study is to compare, in advanced stage, previously untreated CLL patients receiving 4 cycles of fludarabine, the effi-
cacy of high-dose chemotherapy followed by autologous transplantation with PBSC versus 2 further cycles of fludarabine alone in maintaining the clinical (and the possible biological) remission.

The study is a prospective, randomized, multicenter phase III study. The primary end-point is progression-free survival (PFS) and the secondary end-points are: response after 4 cycles of fludarabine (before randomization), response after cyclophosphamide (arm A), response at the end of the assigned treatment, treatment-related toxicity, and overall survival (OS). Different biological end-points have also been set: the number of progenitors mobilized following cyclophosphamide and granulocyte colony-stimulating factor (G-CSF), and the number and duration of molecular and immunophenotypic remissions.

All previously untreated CLL patients aged <60 years in advanced stage of disease are eligible. For patients <45 years with an HLA-identical donor, inclusion into the study depends on the decision of the managing center. A total number of 300 patients is expected to be enrolled with a planned recruitment period of 3 years.

### Treatment plan

Following cytoreduction with 4 courses of fludarabine, patients with complete (CR) or partial (PR) remission are randomized to receive the following treatment: arm A, mobilization and collection of PBSC after cyclophosphamide 7 g/m² + G-CSF administration, followed by an autologous transplantation (conditioning regimen: mitoxantrone + melphalan), or arm B, based on 2 further courses of fludarabine. Patients with <PR and patients with progressive disease or relapse go off study (Figure 1).

### Clinical evaluation of response

According to NCI criteria.

### Evaluation of toxicity

According to WHO criteria.

### Response criteria

Clinical response is determined following the NCI criteria. For biological response, the following parameters are considered: an immunophenotypic response is defined by the presence in the blood and/or marrow of <10% CD5+/CD19+ (or CD5+/CD19+) cells (evaluated on total lymphocytes) with a κ/λ ratio < 3/1; molecular response is defined by the negativity of the IgH gene rearrangement in blood and marrow lymphocytes.

### Follow-up

Patients are re-evaluated for disease status every month for the first 12 months after transplant or after the last fludarabine course and subsequently every 2 months. Monitoring of minimal residual disease by immunophenotypic and molecular (IgH PCR) analyses is to be carried out on peripheral blood and marrow lymphocytes at 2, 6 and 12 months after transplant or fludarabine during the first year and subsequently every 6 months, in patients achieving a clinical and immunophenotypic CR after the completion of the treatment.

### Results

A total of 73 patients have been enrolled between May 1999 and May 2002 in 20 Centers. Table 1 shows the list of Centers and the number of patients enrolled per Center. The median age of these 73 patients is 53 years (31-60), 54 are males and 19 females. The majority of patients (50) were in stage B at enrollment, 22 were in stage C and 1 patient was defined as having progressive stage A disease. A total of 63 patients are evaluable after the four-cycle induction treatment: 48 (76%) obtained either a complete (11 cases) or a partial (37 cases) response, while 14 (22%) showed stable
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or progressive disease. One patient died from fungal pneumonia. Of the 48 patients eligible for randomization, 3 refused, 22 have been assigned to arm A (high dose) and 23 to arm B. The projected probability of OS is about 90%, with only two deaths so far reported, one from fungal infection and the other from a second malignancy diagnosed one month after registration.

Concerning the mobilization after fludarabine, preliminary data are available for a total of 11 cases. Mobilization has been successful in 7 (63%), with a median of 5.6×10^6/kg (2.6–6.8) CD34+ cells collected after a median number of 2 aphereses. In all 7 cases, an autograft has been performed and engraftment (neutrophils > 0.5×10^9/L) obtained after a median time of 14 days (10–21).

Discussion

Although the GIMEMA protocol started at the end of 1999, the results are still very preliminary and only a few conclusions can be drawn: high-dose therapy followed by PBSC is an alternative therapy for advanced stage CLL patients; the possibility of collecting a sufficient number of hematopoietic precursor cells after fludarabine therapy in patients with sensitive disease has been confirmed, as has the low toxicity of the high-dose therapy given in an early phase of the disease. The major problem encountered with this protocol has been the slow enrollment of patients; the age limit < 60 years may be too restrictive. Adjuvant therapy (e.g., monoclonal antibodies or other drugs) together with fludarabine in an attempt to obtain a status of minimal residual disease is not permitted and new prognostic factors including fluorescent in situ hybridization (FISH), mutation analysis of IgV genes and evaluation of CD38 expression are not considered. In addition, it must be taken into account that in Europe an EBMT randomized prospective trial is currently underway which to some extent (e.g. early phase patients) overlaps with the ongoing GIMEMA protocol.

Table 1. Centers and patients enrolled.

<table>
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<tr>
<th>Center - Principal investigator</th>
<th>Ethical approval</th>
<th>Patients</th>
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<tr>
<td>Ancona – P Leoni</td>
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<td>Milano – M Bregi</td>
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<td>Montefiascone (VT) – M Montanaro</td>
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References


Autografting for patients with chronic lymphocytic leukemia (CLL) has increased significantly over the past years, with 482 cases registered in the EBMT database and a growing number of published single center series.\textsuperscript{1-5} Due to improvements of supportive therapy, the mortality of the procedure is low and well below 10\%, but in most series a steady decline of the event-free survival curve has been observed due to relapses occurring up to 5 years after transplantation, and it is still not clear whether autografting can be curative.

The largest single-center series of patients treated with autologous stem cell transplantation (SCT) for CLL has been reported by the Dana-Farber Cancer Center.\textsuperscript{6} One hundred and fifty-two patients with advanced CLL underwent intensive therapy including total body irradiation (TBI) and cyclophosphamide followed by re-infusion of autologous bone marrow (BM) purged with anti-B-cell monoclonal antibodies and complement. There were eight treatment-related deaths (5\%). With a median follow-up of about 30 months, only 14 patients have relapsed, but a substantial number of patients (63 of 136 evaluable patients) showed persistent disease at a molecular level. It was shown in this study that there was a correlation between relapse after transplantation and (1) degree of B-cell depletion; (2) and persistent negativity of polymerase chain reaction (PCR) results.

Two important pilot studies carried out in Germany and England effectively showed a very important molecular remission rate after autotransplantation but these two studies also found an increasing number of molecular relapses during the follow-up after transplantation. Nevertheless, due to the low toxicity of the procedure, the outcome of autografted patients is characterized by overall survival figures of more than 75\% 3 years post-transplant, generally better than those after allotransplantation, at least in the early post-transplantation years.

At the University of Kiel,\textsuperscript{7} 93 patients with poor-risk CLL received autotransplants: 90 (97\%) were re-infused with purged autologous stem cell grafts (CD34\(^+\)/CD3\(^-\)) following preparation with TBI/cy clophosphamide. The median number of CD34\(^+\) cells mobilized was 4.7 \times 10^6/kg (range, 1.7-28.9). Engraftment led to prompt neutrophil recovery greater than 0.5 \times 10^9/L after a median of 10 days (range, 8-16 days) and platelets over 20 \times 10^9/L after a median of 11 days (range, 8-40 days). The median duration of hospitalization was 15 days (range, 10-50 days). The transplant-related mortality (TRM) was 5\% (range, 1-9%), overall survival 92\% (range, 84-100\%) at 42 months and progression-free survival 87\% at 24 months and 60\% at 36 months. A high percentage of molecular relapses was observed, with 22\% at 24 months and 80\% at 36 months after transplantation. A significant impact of cytogenetics on molecular relapse rate after transplantation was also observed, with a great number of relapses in patients with 11q- abnormality.

The Medical Research Council has been conducting a pilot study\textsuperscript{3} of autografting in CLL since 1996. They entered 119 patients and information is available on 59 patients who have undergone autologous transplantation. Concerning peripheral blood stem cell (PBSC) mobilization, 16 patients had an insufficient number of CD34\(^+\) cells after mobilization, 15 underwent re-mobilization and 8 presented a true failure of mobilization. The median number of CD34\(^+\) cells mobilized after cyclophosphamide and granulocyte colony-stimulating factor (GCSF) was 2.7 \times 10^6/kg (range, 0-6). Of these, 78\%
became PCR negative for IgH CDR III re-arrangements after autografting. All patients who were in morphologic CR at the time of autografting were PCR negative after transplantation and only 5 of 29 of these patients have had a molecular relapse. This study also found an increasing number of molecular relapses during the follow-up after transplantation (Figure 1); nevertheless the survival was excellent in the transplanted patients, with a projected overall survival of over 80% at 3 years. The TRM was about 3%.

The EBMT has recently updated data on the outcome of 482 autologous transplants (autoT) from the EBMT registry (Table 1). There were 381 males (79%) and 101 females (21%), with a median age of 50 years (range, 22 to 66). The median interval between diagnosis and transplantation was 26 months (range, 4 to 215). At diagnosis, 68% of the patients studied were in stage B or C according to the Binet classification. Thirty-five percent of patients had received one conventional line of therapy, 37% two lines, and 28% three lines before autoT. These lines included fludarabine for 126 of 482 patients (26%). At transplantation, 413 patients were evaluated for the response to therapy: 129 of 413 (31%) were in complete remission (CR), 223 of 413 (54%) were in partial remission (PR), and 61/413 (15%) had progressive disease (PD). Three hundred and eighty-three patients (80%) received peripheral blood stem cells. From 170 patients, the PBSCs were mobilized by either chemotherapy alone (4%), G-CSF (11%) or, more frequently, by combining chemotherapy and G-CSF (85%). In addition, 200 of 437 evaluated patients (46%) received a purged graft after negative or positive selection. For the conditioning regimen, 258 of 456 patients (57%) received a total body irradiation (TBI)-containing regimen. After autoT, 440 of 452 patients (97%) engrafted (12 days (range, 1-119) days) to achieve 0.5×10^9/L neutrophils, and 20 days (range, 4-1098 days) to achieve a number of platelets exceeding 50×10^9/L. We evaluated 347 patients for disease response: 279 (80%) achieved a CR, 41 (12%)

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**Table 1. General characteristics of the CLL population receiving autotransplants between 1994 and 2000 (EBMT).**

<table>
<thead>
<tr>
<th>AutoT number (%)</th>
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<tr>
<td>Total number of patients</td>
<td>482</td>
</tr>
<tr>
<td>Female</td>
<td>381 (79)</td>
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<tr>
<td>Male</td>
<td>101 (21)</td>
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<td>Median age</td>
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<td>Range</td>
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<td>Internal diagnosis - transplantation</td>
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<td>≤ 36 months</td>
<td>279 (60)</td>
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<td>&gt; 36 months</td>
<td>188 (40)</td>
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<tr>
<td>Median</td>
<td>26 months</td>
</tr>
<tr>
<td>Range</td>
<td>(4-215)</td>
</tr>
<tr>
<td>Stage at diagnosis (Binet)</td>
<td>216</td>
</tr>
<tr>
<td>A</td>
<td>46 (22)</td>
</tr>
<tr>
<td>B</td>
<td>68 (46)</td>
</tr>
<tr>
<td>C</td>
<td>32 (22)</td>
</tr>
<tr>
<td>Number of therapeutic lines</td>
<td>216</td>
</tr>
<tr>
<td>1</td>
<td>75 (35)</td>
</tr>
<tr>
<td>2</td>
<td>81 (37)</td>
</tr>
<tr>
<td>3</td>
<td>60 (28)</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>26</td>
</tr>
<tr>
<td>Yes</td>
<td>126 (26)</td>
</tr>
<tr>
<td>No</td>
<td>356 (74)</td>
</tr>
<tr>
<td>Status at transplantation</td>
<td>413</td>
</tr>
<tr>
<td>CR</td>
<td>129 (31)</td>
</tr>
<tr>
<td>PR</td>
<td>223 (54)</td>
</tr>
<tr>
<td>PD</td>
<td>61 (15)</td>
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<tr>
<td>Source of stem cell</td>
<td>456</td>
</tr>
<tr>
<td>PB</td>
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<tr>
<td>BM</td>
<td>69 (14)</td>
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<tr>
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<td>30 (6)</td>
</tr>
<tr>
<td>TBI</td>
<td>456</td>
</tr>
<tr>
<td>Yes</td>
<td>258 (57)</td>
</tr>
<tr>
<td>No</td>
<td>198 (43)</td>
</tr>
</tbody>
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AutoT: autotransplantation; AlloT: allotransplantation; PR: partial remission; CR: complete remission; PD: progressive disease; PB: peripheral blood; BM: bone marrow; TBI: total body irradiation.
achieved a PR, and 227 (8%) had stable disease or had progressed. Univariate analysis showed that the projected 3-year survival was 79% (SE=3%) (Figure 2). At 42 months after autoT, 75% of patients were still alive. The projected 3-year TRM was 11% (SE=2%) and the risk of relapse at 3 years was 40% (SE=4%) (Figure 3). After autoT, the risk of relapse increased over time and was higher at 60 months than at 20 months after transplantation. Using log-rank comparisons, we found no significant association between survival and gender or age of recipient. Even though it concerned only a small subset of patients, we found a significant association between survival and stage at diagnosis with the worse survival occurring among stage C patients. In addition, we found a significant association between survival and (1) short interval (≤36 months) between diagnosis and transplantation (p<0.01), (2) status of the disease before transplantation in favor of CR (p<0.01), (3) the number of lines of conventional therapies in favor of one line, (p=0.01), and (4) the conditioning regimen in favor of TBI (p<0.01). Finally, we also showed a trend in favor of PBPC as the source of stem cells (p=0.08). The multivariate analysis considered age and gender of recipient, year of transplantation, type of transplant, interval between diagnosis and transplantation, fludarabine, and CHOP before transplantation, disease status before transplantation, and TBI during conditioning. The results of multivariate analysis demonstrated a significant association between survival and (1) short interval between diagnosis and transplantation (≥36 m vs ≤36 m: HR=2.58) (Figure 4), (2) CR before transplantation (PR vs CR/PD vs CR: HR=2.37/HR=6), and (3) TBI-containing regimens (HR=0.35).

In an analysis from the International Bone Marrow Transplant Registry (IBMT), Esteve et al. demonstrated similar results for 124 autologous transplantations, with 87% CR after transplantation, TRM at 6%, overall survival of 63±7% and a
risk of relapse at 68±9% which was significantly higher than after allogeneic transplantation (23±13%, p<0.001). In a multivariate analysis, the author demonstrated a significant relation between survival and number of therapeutic lines, status of the disease, stage before transplantation, percentage of lymphocytes in the marrow and interval between diagnosis and transplantation.

Although a number of single-center or multi-center SCT studies have been performed or are currently underway, the impact of autologous SCT on the prognosis of CLL is still unclear. To determine the real place of autotransplantation in CLL, we now urgently need a large randomized trial for selected patients.

Myeloablative regimens

Although encouraging results have been observed after high-dose chemotherapy alone followed by autologous SCT, the vast majority of published data on stem cell transplants for CLL describe myeloablative regimens containing TBI because CLL cells are very sensitive to irradiation. On the other hand, it is unlikely, given the results of conventional therapy, that cytotoxic drugs alone can eradicate CLL.\(^8,9\) A retrospective analysis from the EBMT\(^1\) also suggested that TBI-based regimens were superior to chemotherapy, although selection bias could not be discounted as a cause for the difference. Thus, TBI/cyclophosphamide still appears to be the gold standard for autografting patients with CLL, although regimens employing high-dose chemotherapy alone may have similar efficacy.

Stem cell source and PBSC mobilization

Due to their favorable engraftment kinetics, mobilized PBPCs have now replaced bone marrow as the principal source of stem cells. A variety of G-CSF-based mobilization regimens are currently in use. Very preliminary data indicate that the mobilization efficacy of more intensive protocols such as the Dexa-BEAM regimen appears to be somewhat better than that of classical cyclophosphamide plus G-CSF combinations.\(^2\) This superior stem cell yield is, however, at the expense of increased toxicity and cost.

We have performed a retrospective European survey of PBSC mobilization\(^10\) in patients who received fludarabine before transplantation. We did not observe any mobilization problems, with a median of 4.29\(\times\)10^9/kg CFU-GM and 2.2\(\times\)10^6/kg CD34\(^+\) cells. Variables that may influence mobilization efficacy were stage, time from diagnosis, extent of pretreatment (fludarabine alone: 6.3\(\times\)10^6 vs 1.9\(\times\)10^6 fludarabine + other chemotherapy), number of courses of fludarabine \(<6\) courses: 1.9\(\times\)10^6 vs \(\geq 6\) courses: 2.6\(\times\)10^6 (p=0.021) and interval between the last course of fludarabine and the start of mobilization \(<2\) months: 1.5\(\times\)10^6 \(\geq 2\) months: 4.8\(\times\)10^6 (p=0.021). In France, we recently performed a study of PBSC mobilization after combined oral treatment with fludarabine and cyclophosphamide. Thirty-eight patients were analyzed, and 51 mobilizations were followed by 46 apheresis procedures. Seventeen apheresis procedures were done more than 200 days after the last course of fludarabine/cyclophosphamide; the authors observed 7 failures, in 5 cases the CD34\(^+\) cell number was less than 2\(\times\)10^6 and in 5 cases more than 2\(\times\)10^6. In total, in 55% of the cases, mobilization was not possible, in 10% we obtained between 0 and 1\(\times\)10^6 CD34\(^+\) cells, either 1 and 2\(\times\)10^6 CD34\(^+\) cells, or 2 and 3\(\times\)10^6 CD34\(^+\) cells and in 15% more than 3\(\times\)10^6 CD34\(^+\) cells.

Purging

The performance of systems for ex-vivo B cell depletion from stem cell grafts has been further refined during recent years.\(^\text{11}\) With modern CD34\(^+\) cell selection devices such as Isolex 300i or Clini-macs, it is possible to eliminate 3-4 log of CLL cells from fresh leukapheresis products. The purging efficacy can be further increased by incorporating a step of negative B-cell depletion into the procedure.\(^\text{12}\) This maneuver also allows the elimination of presumed CD34\(^+\) CLL cells. In spite of sophisticated purging technology, there is still uncertainty about the clinical benefit of this strategy.

References

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Chronic lymphocytic leukemia: is it a curable disease?


Hematopoietic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplantation for chronic lymphocytic leukemia: background and results from the EBMT Registry

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Conventional treatment of chronic lymphocytic leukemia (CLL) has aimed to prolong survival and improve quality of life. However, the advent of allogeneic hematopoietic stem cell (HSC) transplantation has raised the hope of curing this neoplasm. At present, less than 150 CLL patients worldwide have been reported, in peer-reviewed journals, to have undergone high-dose chemoradiotherapy and BMT, mostly from HLA-identical siblings. Considering that CLL is the most common form of leukemia in the West, and that nearly 100,000 allogeneic HSC transplants have been performed for acute and chronic leukemias, it is clear that the place of HSC transplantation in the management of CLL remains to be defined. Here we examine the rationale for allografting in CLL and then summarize the results of allotransplants performed to date.

Age of patients

The median age of onset of CLL is 60 years, currently beyond the commonly used upper age limit for HLA-identical sibling transplantation of 55 years. However, about 10% of patients with CLL are less than 50 years old at diagnosis. The prognosis of these younger patients is in general much better than that of the general population with CLL; in fact, their median survival was 9 years in one series, was not reached at 5 years in a second study, and was 12.3 years in a survey by the Spanish Co-operative Group for CLL. These patients are candidates for bone marrow transplantation (BMT), although we know that increasing age adversely affects the results of transplantation. The recent enthusiasm for the use of the so-called non-ablative transplants might be particularly relevant to CLL patients, because many of them are too old or too sick for conventional transplants: if it is a successful strategy, there is potential for a multi-fold expansion of these procedures in a large number of patients. A few cases have been reported but their follow-up is generally too brief to draw sound conclusions.

Prognostic factors

The clinical course of CLL is very variable. There are two well-known staging systems, the Rai classification and the Binet classification, which both identify patients at low, intermediate, and high risk. These staging systems can be supplemented with additional data such as lymphocyte count at diagnosis, cytogenetic findings, pattern of marrow infiltration, and lymphocyte doubling time, so that patients at one extreme can be identified with a survival similar to that of an age- and sex-matched control population and those at the other extreme with a median survival of less than 2 years. The decision to undertake allogeneic HSC transplantation and its timing should be made taking into account these prognostic factors. They have been shown to be valid for younger patients. Those with the worst risk should be candidates for an early transplant.

Conventional therapy of CLL

A number of antimitic agents (alone or in combination) are active in CLL. These agents include chlorambucil, cyclophosphamide, vincristine, corticosteroids, melphalan, busulfan, and anthracycline antibiotics. Additionally, CLL lymphocytes are very radiosensitive. A dose-response effect, an ideal prerequisite for the application of HSC transplantation, has been shown in one prospective, randomized study comparing CHOP with COP in advanced CLL; this demonstrated the superiority of the adriamycin-containing regimen. Finally, a new class of drug, - the purine
analogs - has shown promising results and these drugs are likely to play a significant role in the treatment of CLL. In particular, fludarabine has been shown to be the most effective single agent in CLL, both in previously treated and untreated patients. This drug, now widely employed, may well become, alone or in combination, the standard treatment of CLL in the future, but, despite long remissions, the disease is almost invariably followed by relapse.

Evaluation of response to treatment

After conventional treatment a complete response is broadly defined as normalization of blood and bone marrow parameters, resolution of organomegaly, and absence of symptoms. However, these criteria do not apply if the aim of treatment is elimination of the neoplastic clone. More stringent criteria of complete remission include the normalization of T- and B-cell numbers and the \( \kappa / \lambda \) light chain ratio, and the presence of very low numbers of cells phenotypically characteristic of B-CLL (for example CD5+ CD20+) by dual marker analysis. Finally, direct evidence of the eradication of CLL can be provided by the absence of markers of the neoplastic clone; immunoglobulin gene rearrangement studies can detect low numbers of CLL cells (1% to 2%) if the individual patient's pattern of rearrangement is known. Using molecular biology techniques, such as polymerase chain reaction amplification of the rearranged immunoglobulin heavy chain locus (IgH), it is possible to detect residual tumor at a much lower threshold level. This type of analysis is well suited to assess the patients' status after transplantation.

Results of HLA-identical sibling transplantation. Analysis of peer-reviewed literature

There are now a few reports from single institutions involving small numbers of patients and a single, relatively large, multicenter study. This analysis, from the International Bone Marrow Transplant Registry and the European Group for Blood and Marrow Transplantation, described 54 patients who underwent HLA-identical sibling bone marrow transplantation. Thirty-nine patients were able patients. Engraftment of neutrophils and platelets occurred within the usual time frame. Of the 45 who engrafted, 2 had a late (7 and 12 months) autologous reconstitution: both had received a T-cell-depleted graft. The disappearance of splenomegaly/hepatomegaly and lymph node enlargement usually occurred between the 1st and 3rd week, while blood lymphocytosis decreased slowly, generally between the 3rd and 4th week.

Stable engraftment occurred in 45 of 49 evaluable patients. Engraftment of neutrophils and platelets occurred within the usual time frame. Of the 45 who engrafted, 2 had a late (7 and 12 months) autologous reconstitution: both had received a T-cell-depleted graft. The disappearance of splenomegaly/hepatomegaly and lymph node enlargement usually occurred between the 1st and 3rd week, while blood lymphocytosis decreased slowly, generally between the 3rd and 4th week.

However, normalization of the lymphocyte count took a long time (several months) in some patients, as we and others have observed after syngeneic or allogeneic transplantation. Acute GVHD was absent in 10 patients, was grade I in 20, grade II in 6, grade III in 6, and grade IV in 5. Of 35 patients at risk, 17 developed chronic GVHD: it was extensive in 6 and limited in 11. Thirty-eight patients achieved hematologic remission; five relapsed after 4, 7, 11, 48, and 58 months. Three of the five relapses occurred in recipients of T-cell-depleted transplants and two in recipients of unmanipulated transplants. In four patients studies of the immunoglobulin JH region rearrangement were performed before and after BMT on peripheral blood lymphocytes; the rearrangements demonstrated pretransplant were no longer present. Thirty patients died after BMT: the cause of death was relapsed CLL in 5 cases and treatment-related in 25: failure of engraftment 4, acute GVHD 6, chronic GVHD 4, cerebral hemor-
rhage 1, infection 4, hepatic veno-occlusive disease 4, and interstitial pneumonitis 2. Twenty-four patients were alive at the time of the report, of whom 23 were in hematologic remission, at a median of 27 (range 5–80) months post-transplant. Three-year survival probability was 46% (CI, 32%–60%). There was no statistical difference in survival for patients transplanted in stages 0–III (stage 0: 100%; stage I: 68%, CI, 38%–98%; stage II: 30%, CI, 2%–58%; stage III: 57%, CI, 21%–93%). Patients with stage IV disease had a 34% (CI, 12–56%) probability of 3-year survival. Three-year probability of survival was 86% (CI, 62–100%) in patients with responsive disease; 61% (CI, 38–84%) in those with stable disease, and 23% (CI, 2–44%) in those with progressive disease.

Transplants have also been performed in advanced disease. Rodriguez et al. reported on 8 patients with Richter's syndrome (CLL transformed to an aggressive lymphoma, usually diffuse large cell lymphoma). At the time of transplantation all had been heavily pretreated, 2 had failed to respond to autologous transplantation, 5 had refractory disease, 5 had B symptoms and 7 had stage III or IV disease. At the time of the report, 3 were alive and in remission at 14, 47, and 67 months post-transplant. Two of these three had received non-myeloablative regimens.

A small number of cases of prolymphocytic leukemia have been treated by allogeneic HSC transplantation. Of the four cases reported in the latter study, three were alive and in complete remission at the time of the report at >2, >11 and >24 months post-transplant. Two received myeloablative conditioning and a sibling transplant, one non-ablative conditioning and a sibling transplant, and one non-ablative conditioning and an unrelated donor transplant. Another case of unrelated transplant was reported by Toze et al., in a larger series of CLL transplants. The patient, a 46-year old male achieved a CR after transplant but died of progressive multifocal leukoencephalopathy after 13 months. A patient with refractory PLL, who received a non-ablative regimen, was recently reported. The patient relapsed at day 84 and died of progressive disease at five months, despite donor lymphocyte infusions (DLI) and chemotherapy.

Results of HLA-mismatched family member transplantation

Three cases of 1 or 2 antigen family mismatched grafts have been reported in a larger series of CLL transplants. All had refractory disease, received myeloablative TBI/cyclophosphamide-based regimens and cyclosporine/methotrexate or tacrolimus/methotrexate for GVHD prophylaxis. Two patients were alive (one after a second transplant), with no evidence of disease, at the time of writing, while the third had died of GVD and infections after DLI. Another patient with refractory disease received the marrow from a DRB1 mismatched sibling, after a 200 cGyTBI non-ablative regimen. He died of GVHD on day 112, with no evidence of disease by immunophenotypic analysis.

Results of unrelated donor transplantation

Few cases have been reported. One patient, with progressive disease, received a partially mismatched unrelated transplant, which was T-depleted; the post-transplant course was uneventful and he was alive, in remission, at 10 months. One patient with refractory disease received a conventional transplant and was alive in remission, with limited chronic GVHD, at the time of writing. A third patient was reported, alive without evidence of disease, two years after a T-cell-depleted transplant.

Current data of the EBMT registry

Two-hundred and nine allogeneic BMT, performed between 1994 and 2000 have been registered with the EBMT. There are 163 males (78%) and 46 females (22%), with a median age of 42 years (range, 22 to 64). The median interval between diagnosis and transplantation was 45 months (range, 5 to 198). The general characteristics of the population are described in Table 1. At diagnosis, the majority of the patients studied were in stages B or C according to Binet’s classification: (76%). Twenty-two percent of patients had received one conventional line of therapy, 28% two lines, and 50% three lines of treatment before their transplant. These lines included fludarabine for 44 patients (21%). Moreover, 30 (14%), had received chlorambucil-containing treatment and 32 (15%) chemotherapy consisting of cyclophosphamide, vincristine, adriamycin and prednisone (CHOP). At transplantation, 172 patients were evaluated for the response to therapy: 19 patients (11%) were in complete remission (CR), 78 (45%) were in partial remission (PR), and 75 (44%) had progressive disease (PD).

Ninety patients (43%) received BM, 115 PBSCs (55%) and 4 BM and PBPCs (2%). One hundred and sixty-six patients (83%) received an allograft from HLA-identical sibling donors, 6 from syngeneic donors (3%), 16 (8%) from matched and mismatched related donors, and 12 (6%) from unrelated donors. For the conditioning regimen, 64% received a TBI-containing regimen and 22% received cyclophosphamide/alkylators.
received a T-depleted graft for graft-versus-host disease prophylaxis.

**Results**

Engraftment and graft-versus-host disease

One-hundred and eighty-three of the 197 patients engrafted (93%), requiring 16 days (range, 0 to 100) to achieve a number of 500 PMN/m³ and 25 days (range, 0 to 214) to achieve a number of platelets greater than 50,000/m³. Sixty-five patients (34%) out of 190 who were evaluable developed acute GVHD ≥ grade 2 and 47 patients (49%) out of 95 who were evaluable developed chronic GVHD (28 limited and 19 extensive).

One hundred and forty-one patients were evaluated for response of the disease: 101 patients (72%) achieved a CR, 19 patients (13%) achieved a PR, and 21 patients (15%) were in stable disease or had disease progression.

Factors predicting transplantation outcome

Univariate analysis

The projected 3-year survival was 55% [SE=5%] (Figure 1). At 5 months after transplant 75% of patients were alive. The projected 3-year transplant-related mortality (TRM) was 40% [SE=5%] and the risk of relapse at 3 years was 27% [SE=7%].

Using log-rank comparisons, we found no significant association between survival and gender or age of recipient. Although it applied to only a small subset of patients, we found a significant correlation between survival and stage at diagnosis with a worse survival for stage C patients. Regarding conventional therapy before transplantation, we could not prove any survival difference after BMT between patients treated with CHOP and other therapies. We did, however, find a significant association between survival and fludarabine (p=0.03) and survival and chlorambucil (p=0.02) given before transplantation. Finally, we showed a significant association between survival and stem cell source in favor of PBPCs (p=0.05).
Multivariate analysis

The multivariate analysis considered age and gender of recipient, year of transplantation, type of transplant, interval between diagnosis and transplant, fludarabine, and CHOP before transplantation, disease status before transplantation, and TBI during conditioning. The results of multivariate analysis demonstrated a significant association between survival and fludarabine (HR=0.48), and year of transplantation (HR=0.87). In addition, using a Cox model for lines of therapy and stage at diagnosis, the HR was in both cases highly non-significant and showed a clinically irrelevant difference. Moreover, by considering the interaction between fludarabine used and stage at diagnosis known or unknown, we verified that the estimated effect on survival of fludarabine did not depend on the missing data on stage at diagnosis. Hence we conclude that any analysis on the subset of patients with these two known risk factors is representative for the entire population.

Conclusions

The number of patients so far reported is too small to allow in-depth statistical analysis. However, from the analysis of the largest reported group of CLL patients treated by HLA-identical sibling BMT18 and from the preliminary analysis of the EBMT data, a few observations can be made. Firstly, allogeneic BMT can be successfully performed in CLL, resulting in long-term leukemia-free survival in patients with either early or advanced disease; the main cause of death were transplant related and occurred early after BMT. A similar, although slightly lower transplant-related mortality was reported by Pavletic et al.;6 of 23 patients, 8 died of transplant related cause and only one of disease. These data contrast with the low mortality reported in some small studies from single institutions. At the Dana-Farber Cancer Institute, eight patients with CLL were allotransplanted in complete remission, having responded to fludarabine: seven of them were alive with a follow-up period ranging from 6 to 18 months.3 Of six patients who received HLA identical sibling transplants at the Vancouver Hospital, two died, one of infection and one of relapse.7 Of 12 patients transplanted at the Hospital Clinic in Barcelona, 3 died of transplant-related cause and only one of disease. These data contrast with the low mortality reported in some small studies from single institutions. 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Chronic lymphocytic leukemia: is it a curable disease?

A number of patients of 15 patients with advanced disease, 7/15 died after transplant, in only one case due to disease progression and in five of transplant-related causes. It is matter of speculation whether the prior use of fludarabine, patient selection, or other factors contributed to the low incidence of acute GVHD and overall transplant-related mortality in the earlier reports. However, these data are encouraging and it can be expected that transplant-related morbidity and mortality will, in the future, be reduced in CLL patients similarly to what has been observed in other malignancies, especially the acute leukemias, during the past decade.30

A second point of note is that overall survival was best in patients with Rai stages 0 to III, and in those with stable or responsive disease. Such findings are analogous to results in most hematologic malignancies. Patients with more advanced CLL, and more prior therapy, are more likely to have a poorer performance status at transplant than those with less advanced disease. A report from the Royal Marsden Hospital has underlined the occurrence of unusual opportunistic infections in six CLL or PLL patients following allogeneic BM T.31-32 Thus, it is likely to be advantageous to transplant earlier in the course of the disease, soon after a stable decrease of the leukemic mass has been achieved. It is of interest in this respect that the three patients transplanted in Rai stage 0 became long-term survivors. The incidence of severe acute GVHD was high, with 10 patients having grade III-IV disease; this finding could perhaps have been expected in view of the median age of the population.33

Finally, these data indicate that HLA-identical sibling BM T is an effective, curative treatment for refractory CLL, with a 3-year relapse risk of about 30% (Figure 2). A complete disappearance of the neoplastic clone could be documented with molecular biological techniques in four patients; in our view, these latter findings meet the criteria for complete remission discussed above. Another study on the clinical usefulness of molecular methods to assess residual disease after high-dose therapy described three patients with CLL who were persistently polymerase chain reaction-negative for 4 years after HLA-identical sibling BM T.18 A similar outcome was described in a single patient by Sar-doun et al.34

Recently, employing the exquisitely sensitive analysis of the rearrangement pattern of the CDRII region of the heavy chain immunoglobulin (IgH) gene, Esteve et al.5 found 64% negativity for minimal residual disease (MRD) after 14 cases of autologous transplantation, but over the course of a long follow-up, two of the nine patients had a clinical relapse and four became MRD positive. In contrast, of the 8 allotransplants who achieved a clinical CR, all became MRD negative (in two patients requiring up to 22 months to do so) and none had a MRD or clinical relapse.35 These data suggest that the type of response achieved after allogeneic transplantation is durable, and that the procedure may result in true cure in some cases and that this is related to the allogeneic effect of the graft, be it coincident or not with GVHD. This is in contrast to what is seen after chemotherapy or after autologous transplant, after which the disease eventually always recurs, although some of the remissions may be quite long.

Other patients in the IBMTR-EBMT study have no sign of disease, with follow-up times ranging from 6 to 80 months; five patients have follow-up times greater than 5 years. One of them received BMT as the initial treatment of CLL, but the others had advanced disease at the time of transplant. However, two late relapses occurred at 48 and 58 months, so it is necessary to be cautious in interpreting long-term results. Interestingly, there are at least five reports suggesting that a graft-versus-leukemia effect is operative in this malignancy and in the related PLL.9,36-39 In these reports, disease remission occurred after withdrawal of immunosuppression, infusion of donor lymphocytes or the onset of GVHD. Also the delayed clearance of CLL, taking as long as one year or more in a few patients,520,34 has been
explained by modulation of the graft-versus-tumor effect on residual leukemic cells.

The existing data indicate that the choice of allogeneic transplantation should be based on the disease risk, on the one hand, and the transplant risk, on the other. In patients with high-risk CLL, allogeneic transplants should be considered as first-line treatment if the transplant-related mortality risk is not high - younger patients with no co-existing infections or other co-morbidities, younger donors, perfect HLA matching. In the remaining cases, allogeneic transplantation should be employed as a second- or third-line therapy. Since there are several reports of response even in cases of advanced disease, the deferral of transplantation would not be detrimental and the increased risk of transplant-related mortality would thus be justified by the disease status. Clearly, a good balance between disease risk and transplant risk is not easy to assess or determine in everyday clinical practice, but, nonetheless, it should be the logical way to guide the physician in selecting the therapeutic strategy for each individual patient.

We think that further studies of the use of allogeneic HSC transplantation in selected patients with CLL are justified and should be encouraged, with the aim of better defining the indications and timing of transplant, while taking into account the availability of the newer chemotherapy agents. Such studies should help to establish the precise role of allogeneic transplantation in the management of CLL.

References


Prolonged remissions have been reported in patients with advanced chronic lymphocytic leukemia (CLL) whose disease did not respond to fludarabine-based therapy but who received high-dose chemotherapy and allogeneic bone marrow or peripheral blood progenitor cell transplantation. Case-control studies have demonstrated that such patients have a superior outcome to patients treated with autologous transplantation or further conventional chemotherapy. This finding was further confirmed recently in a study from the International Bone Marrow Transplant Registry. Evidence supporting a graft-versus-leukemia (GVL) effect in patients with CLL has been demonstrated in studies in which remission was induced through the modulation of immunosuppressive agents, donor lymphocyte infusion, and successful conversion from a positive polymerase chain reaction (PCR) status early after transplantation to a negative PCR status over time. These findings suggested to us that non-ablative allogeneic cell stem transplantation may be an effective alternative therapy for CLL.

Patients were eligible for our study if they were refractory or failed a prior response to fludarabine. Eighteen patients were treated in 2 consecutive trials. All patients received a preparative regimen of fludarabine (30 mg/m² daily for 3 days) and intravenous cyclophosphamide (300 to 750 mg/m² daily for 3 days). Nine patients received rituximab in addition to fludarabine and cyclophosphamide. The median age of the patients was 55 years. Nine of 18 patients had disease that was refractory to fludarabine. The median number of prior chemotherapy regimens received per patient was 3. The use of rituximab enhanced engraftment of donor cells and early tumor control to allow time for the graft-versus-leukemia effect to become established. The median percentage of donor cells at one month post-transplantation was 93 (range, 70 to 100) for the patients who received rituximab with their preparative regimen versus 50 (range, <10 to 100) for the remaining patients.

Eight patients required immunomanipulation with or without rituximab after their transplants. Five patients had a complete response and one had a partial response; 5 of these 6 patients had received rituximab. Survival (72%) and progression-free survival (59%) at one year were comparable to those of a historical control of patients with a median age of 42.6 years (range, 26-57.7 years) who received high-dose chemotherapy and allogeneic transplantation at our institution.

Our results further confirm the graft-versus-leukemia of allogeneic stem cell transplantation in patients with chronic lymphocytic leukemia. The comparative outcome of patients treated with high-dose chemotherapy suggests that non-ablative allogeneic cell stem transplantation is preferable, especially in patients with chemosensitive disease.

References

Chronic lymphocytic leukemia: is it a curable disease?


Only valid role is in a research setting
I do not believe that our experience with stem cell transplants in chronic lymphocytic leukemia (CLL) is large enough and objectively proven enough that we can recommend this method of treatment in routine practice. I do, however, believe that stem cell transplantation has a legitimate and valid place in the treatment of CLL, if it is pursued in a controlled clinical investigational setting. Although it is praise-worthy that today we are devoting an entire international congress to exploring whether we can cure CLL, a sobering, reality check also deserves to be introduced into these deliberations.

When it comes to transplants, why are chronic myelocytic and chronic lymphocytic leukemias different?
Aside from the known existence of several cases of long-term survivors from bone marrow transplantation therapy in acute lymphocytic leukemia and acute myelocytic leukemia, the most widely accepted and non-controversial role for this method of treatment has certainly been established in the chronic phase of chronic myelocytic leukemia (CML), and with an HLA-compatible sibling as the donor. Experience dating back nearly 3 decades, and investigations carried out by experts in Europe and USA, have both shown that compatible sibling donor-derived stem cell transplants in chronic phase CML emerged gradually and progressively (rather than in an abrupt, overnight manner). The pathognomonic feature of CML, the Philadelphia chromosome t (9; 22) involving bcr/abl provides a reliable marker to determine whether the disease is in a true complete cytogenetic (or molecular) remission, as contrasting with a complete clinical (or hematologic) remission. In CLL, however, we do not have a discrete molecular or cytogenetic marker which is accepted as pathognomonic of the disease. We all agree that flow cytometry can reveal whether the CD5 and CD23 co-expressing CD19+, CD20+ monoclonal B-cells have been eliminated from the blood and bone marrow. Such cases can be considered to represent phenotypic or immunologic remission, which is certainly a major step beyond a complete remission (CR) defined by clinical criteria. Nevertheless, an immunologic or phenotypic remission does not assure us that a cytogenetic remission also has been achieved or that immunoglobulin VH gene mutation status has been transformed to a type which is associated with a favorable prognosis.

Progress in CLL
We must recognize, however, that we have made important progress in CLL just within the past decade. Whereas, even by merely clinical criteria, we were not able to induce CRs in more than 5% of previously untreated case of CLL, this proportion increased to 20% with the use of single-agent fludarabine, and to 40% when rituximab was added to fludarabine, and to more than 60% by further adding cyclophosphamide.

Now that we are succeeding in increasing the (clinical) CR rates in this disease, it is appropriate to start examining whether these patients are also achieving a phenotypic remission, and then eventually we might also attempt to seek cytogenetic remissions. In the context of this level of progress, it is now necessary to engage in well-planned research questions concerning the role of stem-cell transplantation.

Transplants in CLL: which cases? when? what type?
Which cases?
A. In order to help to define the beneficial role of transplants in CLL clearly, we must first identify a subpopulation of patients with this disease with as much homogeneity as is practical to con-
duct a prospective, controlled clinical trial. The population which is most likely to benefit consists of young patients who are not previously heavily treated and have chemo-sensitive disease. It would be ideal if all these cases were also those whose leukemic B-cells did not co-express CD38 and who had IgVH gene hypermutations, and a favorable cytogenetic profile.

B. A separate study should be planned for the young patients who have previously been heavily treated or have chemo-resistant disease. I would expect that these patients would also have unfavorable cytogenetic/mutation/CD38 profiles.

What type?
We recognize that a syngeneic transplant from an identical twin is not a practical solution, but in CLL the second choice would be an HLA-matched sibling with peripheral blood stem cells which have been harvested after growth factor priming. But in reality, it is unlikely that more than a minority of patients will have HLA-matched sibling donors available. A non-related matched donor is not recommended for CLL patients because the morbidity and mortality rates in such situations are very high. If a compatible sibling donor is not available, an alternative treatment described below might offer a comparative regimen. It might be appropriate to offer these patients one of the following two treatment strategies - one with autologous peripheral blood stem cells and the other with alemtuzumab as an alternative to transplant. In the autologous setting although the Dana Farber group in Boston uses in vitro purging, most others do not, and I would side with the non-purging group.

When?
I would not try to sell the idea of a transplant to a patient as an already proven, effective treatment. I would clearly spell out to each patient that this treatment is entirely in the investigational domain. If a patient understands this, is able to comprehend the risks and benefits and is able to make a decision whether or not to enroll in this research, I would plan a transplant early in the course of treatment and not wait until the patient has been heavily treated or has become refractory to chemotherapy.

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