Results of the MRC pilot study show autografting for younger patients with chronic lymphocytic leukemia is safe and achieves a high percentage of molecular responses

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We have assessed autologous stem cell transplantation after treatment with fludarabine in previously untreated patients with chronic lymphocytic leukemia (CLL). This study is the first to enroll previously untreated patients and follow them prospectively. The initial response rate to fludarabine was 82% (94 of 115 patients). Stem cell mobilization was attempted in 88 patients and was successful in 59 (67%). Overall 65 of 115 patients (56%) entered into the study proceeded to autologous transplantation. The early transplant-related mortality rate was 1.5% (1 of 65 patients). The number of patients in complete remission after transplantation increased from 37% (24 of 65) to 74% (48 of 65), and 26 of 41 patients (63%) who were not in complete remission at the time of their transplantation achieved a complete remission after transplantation. The 5-year overall and disease-free survival rates from transplantation were 77.5% (CI, 57.2%-97.8%) and 51.5% (CI, 33.2%-69.8%), respectively. None of the variables examined at study entry were found to be predictors of either overall or disease-free survival. Sixteen of 20 evaluable patients achieved a molecular remission on a polymerase chain reaction (PCR) for immunoglobulin heavy-chain gene rearrangements in the first 6 months following transplantation. Detectable molecular disease by PCR was highly predictive of disease recurrence. It is of concern that 5 of 65 (8%) patients developed posttransplant acute myeloid leukemia/myelodysplastic syndrome. (Blood. 2005;105: 397-404)

Introduction

Chronic lymphocytic leukemia (CLL) is characterized by the gradual accumulation of small mature B lymphocytes in the blood, bone marrow, and lymphoid organs. Alkylating agents such as chlorambucil, either alone or with steroids, have traditionally been the mainstay of treatment for patients with advanced stage disease,[1] but do not appear to improve overall survival. Combination chemotherapy,[1-5] particularly with the addition of an anthracycline,[3,6,7] has also been effective at palliating disease. More recently the nucleoside analogues, in particular fludarabine, have induced good responses both in previously untreated patients[7-9] and in patients who are refractory to standard therapy.[10-12] When fludarabine is used to treat previously untreated patients, it is demonstrably better than chlorambucil,[9] and at least as effective as CHOP[7] (cyclophosphamide, hydroxydaunorubicin, Oncovin [vincristine], prednisone) in terms of responses obtained. Evidence indicates that the duration of remissions induced by fludarabine in previously untreated patients is longer,[9,12] although this has not yet been shown to translate into a benefit in overall survival. Fludarabine is also an effective agent when used as second-line therapy[11,12] and is more effective in this setting than combination chemotherapy such as CAP (cyclophosphamide, Adriamycin [doxorubicin], prednisolone).[7] Recently there has been considerable interest in antibody-based approaches[13-19] both alone and in combination. These can result in excellent responses in some patients, with a proportion becoming negative for molecular markers for CLL.[20]

A significant number of patients with newly diagnosed CLL are relatively young with up to 20% being under 55 years of age.[21,22] Although the overall median survival for these patients is approximately the same (10 years) as for the older cohort, they are far more likely to die as a result of CLL,[22] particularly in the presence of adverse prognostic features. These are advanced clinical stage, extensive marrow involvement, high lymphocyte count and short doubling time, CD38 positivity, and adverse genetic abnormalities (del 11q, del 17p, and/or unmutated status of the variable region of the immunoglobulin heavy-chain gene).[21,23-29] Approximately 60% of younger patients with CLL have progressive disease and this group has a median survival of 5 years from therapy.[22] In the United Kingdom Medical Research Council (MRC) CLL-1 and CLL-2 trials[30] the median survivals for patients younger than 55 years with stages B and C disease were 6.5 and 3.5 years, respectively, and thus it is this group that might benefit from more intensive approaches aimed at prolonging survival.

With adequate supportive care autologous stem cell transplantation has been feasible in patients with CLL and results in long-lasting clinical and molecular remissions.[31-34] The transplant-related mortality is less than 10%,[32,33,35] with complete response

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rates in the region of 80%,[31] and possibly some prolongation of survival compared with conventional therapy.[36] A more recent retrospective risk-matched comparison between patients receiving conventional treatment versus sequential high-dose therapy with autologous stem cell transplantation showed a survival benefit for the group receiving transplants, which remained significant when the analysis was restricted to patients with an unmutated IgV_H gene.[37] In addition, high-dose treatment leads to a significant number of molecular remissions.[33,38] However it is also increasingly apparent that the majority of patients attaining complete clinical remission after an autograft will eventually have a relapse.[39] Hence, it is still not known whether autologous transplantation for CLL is associated with extended disease-free survival, at least in a proportion of patients.

Although a number of studies have addressed both autologous and allogeneic transplantation in the management of younger patients with CLL, unanswered questions remain.[31,35,40-43] Previous studies have examined autologous transplantation used as salvage therapy or published data only on patients who received an autograft. There are also concerns about the ability to mobilize adequate numbers of progenitor cells in patients with CLL, particularly after fludarabine therapy. We prospectively analyzed patients, under the age of 60, with newly diagnosed disease requiring treatment for CLL and undergoing autograft. The purpose of this study was to assess the response to fludarabine as a first-line chemotherapeutic agent, to evaluate the feasibility of progenitor cell mobilization after this treatment, and finally to assess the safety and efficacy of autologous stem cell transplantation in this cohort of patients.

Patients and methods

This prospective pilot study involved 31 participating centers from the United Kingdom and New Zealand (see "Appendix"). Local research ethics committees of each center approved the study protocol. All eligible patients were entered into the study after a thorough explanation of what the study involved and gave written informed consent.

Patient characteristics

Patients aged 60 years and younger with previously untreated CLL requiring treatment for stage B or C or progressive stage A disease were recruited between June 1996 and May 2001. Progressive stage A CLL was defined as any of the following: a lymphocyte doubling time of less than 12 months, systemic symptoms, a downward trend in hemoglobin levels or platelet counts, or a localized increase in troublesome lymphadenopathy. Patients were excluded if they were pregnant or lactating, were hypersensitive to fludarabine, had a positive Coombs test, were unable to give informed consent, or had significant comorbidity in the form of serious cardiac, respiratory, renal, or hepatic dysfunction that would prejudice high-dose therapy.

Initial treatment

Eligible patients were treated with fludarabine phosphate 25 mg/m^2 intravenously daily for 5 days, with further courses repeated at intervals of 28 days. A dose modification for subsequent courses was suggested if cytopenias occurred that were felt to be related to treatment. A 50% reduction in dose was used if the neutrophil count was less than 1×10^9/L and platelet count less than 50×10^9/L. If marked neutropenia ($< 0.5 \times 10^9$/L) or thrombocytopenia ($< 20 \times 10^9$/L) occurred, the treatment was temporarily deferred until the blood count improved.

The aim of treatment was to achieve a major response before proceeding to a stem cell harvest. Responses were defined as complete response (CR), partial response (PR), nodular partial response (nPR), and no response (NR). A major response for the purpose of proceeding to attempt peripheral progenitor cell mobilization was defined as the following: regression of clinical lymphadenopathy to less than 2 cm in dimension, no clinical hepatosplenomegaly, no systemic symptoms, no cytopenias, and peripheral blood lymphocyte counts less than 5×10^9/L and bone marrow lymphocytes less than 40%. An nPR on bone marrow trephine examination was permitted.[44]

Progenitor cell mobilization

Progenitor cell mobilization was attempted at least 12 weeks after the last course of fludarabine. Mobilization was performed using cyclophosphamide with granulocyte colony-stimulating factor (G-CSF) although G-CSF alone could be used if desired. The suggested protocol was cyclophosphamide 2 g/m^2 intravenously on day 1 with mesna 1 g/m^2 intravenously or orally at 0, 4, and 8 hours after cyclophosphamide. G-CSF was administered subcutaneously in a dose of 10 μg/kg commencing on day 5 and continuing until the harvest at days 12 to 14. Apheresis was performed when the peripheral white blood cell count was more than 4×10^9/L. A backup marrow harvest was also performed when there were doubts as to the adequacy or quality of the stem cells mobilized. CD34$^+$ cell purification or negative selection of lymphocytes could be performed if desired, using one of the commercially available systems.

Transplantation procedure

The recommended conditioning regimen was cyclophosphamide 60 mg/kg/d intravenously on 2 successive days, together with total body irradiation (TBI), using either 1440 cGy in 8 fractions over 4 days or 1200 cGy in 6 fractions over 3 days. Institutional protocols for emesis control, prophylaxis and treatment of infections, and blood product support were followed. Transplantation procedures were performed at 9 transplantation centers.

Patient monitoring

Patients were assessed for clinical and hematologic evidence of relapse after transplantation with the frequency of follow-up dictated by clinical need. Where possible patients were also assessed for disease relapse using flow cytometry for CD5/CD19 with double staining and gating on the B cells (CD19$^+$), and a polymerase chain reaction (PCR) for immunoglobulin heavy-chain gene rearrangements. The time points for immunophenotyping and molecular studies were at the time of harvest, then 3, 6, 12, 18, and 24 months after transplantation, and annually thereafter.

FR3 PCR method

Samples were amplified by fluorescent IgH PCR using consensus framework 3 primer sequences. Each reaction mixture contained 1 μg DNA, 10 pmole of each primer, 2.5 μL PCR buffer, 1.25 μL 25 mM $MgCl_2$, 0.125 μL 25 mM dNTP, and molecular biology-grade water to make a reaction mixture up to 25 μL. Primer sequences used were Hex-labeled consensus JH primer –ACCTGAGGAGACGGTGACCAGGGT framework 3 consensus primer CCGAGGACACGGC(C/T)(C/G)TGTATTACTG. Following initial heating of the sample to 95°C 1 U *Taq* polymerase (Supertaq, HT Biotechnology, Cambridge, United Kingdom) was added. PCR products were amplified using cycling conditions of 95°C for 60 seconds followed by 60°C for 90 seconds for a total of 35 cycles with a final extension at 72°C for 10 minutes. PCR products were resolved against standard size markers on a polyacrylamide gel using a Genescan 373 automated DNA sequencer (Applied Biosystems, Foster City, CA). For presentation samples clonal rearrangements were identified as a single peak with no polyclonal background. A follow-up sample was considered positive when a peak of identical size to that seen at presentation was found and was at least twice the height of adjacent peaks. The sensitivity of the technique was between 10^{-3} and 10^{-4}.

Minimal residual disease (MRD) was also investigated by flow cytometry by double immunostaining with fluorochrome-conjugated monoclonal antibodies to CD19 and CD5 and gating on the CD19$^+$ cells. A level of more than 15% CD19$^+$/CD5$^+$ cells in the bone marrow and more than 25% CD19$^+$/CD5$^+$ cells in the peripheral blood was considered positive for

MRD on the basis of previous results from normal samples and samples from patients with CLL.[45]

Statistical analysis

Analysis to identify predictors of adequate stem cell harvest was by standard logistic regression. Analysis of survival, disease-free survival, and molecular recurrence was by the Kaplan-Meier method and proportional hazards models were used to investigate the predictive effect of study entry variables on survival and recurrence.

Results

Patient characteristics

A total of 117 patients were recruited (89 men, 28 women) with a median age of 49 years (range, 27-60 years). At study entry, 27 patients (23%) were in progressive Binet stage A, 68 (58%) in stage B, and 22 (19%) in Binet stage C. A summary of patient characteristics at study entry is detailed in Table 1.

Response to initial treatment

Of 117 patients entered into the study, adequate follow-up data were available on 115 (Figure 1). Two patients received just one course of fludarabine (one died of transfusional graft-versus-host disease and the other developed immune thrombocytopenia); hence, 113 were evaluable for the response to fludarabine as a first-line agent. Of these 113 patients who received at least 3 cycles of fludarabine, 94 (83%; CI 75%-89%) responded; 22 (19%) were CRs, 37 (33%) were nPRs, and 35 (31%) were PRs. Forty-four of 115 patients (38%) required further treatment with alternative agents in an attempt to optimize disease control prior to attempting progenitor cell mobilization. The most frequent agents used were cyclophosphamide in combination with fludarabine (12 patients), alemtuzumab (9 patients), and CHOP (7 patients) with occasional patients receiving other combinations. Information was lacking on 7 of 115 patients (6%). The addition of other treatments to fludarabine resulted in a CR in 27 of 115 (23%), nPR in 48 of 115 (42%), and a PR in 27 of 115 (23%). Only 12 of 115 (10%) showed no response to treatment at all and were categorized as primary nonresponders. One patient (1%) died during initial treatment.

Table 1. Characteristics of 117 patients at study entry

Median age, y (range)	49 (27–60)
Sex	
Male (%)	89 (76)
Female (%)	28 (24)
Hematologic parameters	
Median hemoglobin level, g/dL (range)	13.4 (5.5–16.4)
Median white blood cell count, × 10^9/L (range)	80 (3–473)
Median platelet count, × 10^9/L (range)	166 (13–344)
Lymphadenopathy, no. of patients	
None	17
Cervical only	10
Axillary only	5
Inguinal only	0
Cervical and axillary	27
Cervical and inguinal	3
Axillary and inguinal	5
Cervical, axillary, and inguinal	49
Splenomegaly, no. of patients	
None	59
Less than 10 cm	40
More than 10 cm	15
Splenectomy	2
B symptoms, no. of patients	
Yes	77
No	39
Lymphocytes in bone marrow, no. of patients	
Less than 50%	8
50%–75%	19
More than 75%	64
Bone marrow exam not performed	26
ECOG performance status, no. of patients	
0	98
1	11
2	2
Not specified	6
Stage of disease, no. of patients (%)	
A (progressive)	27 (23.1)
B	68 (58.1)
C	22 (18.8)

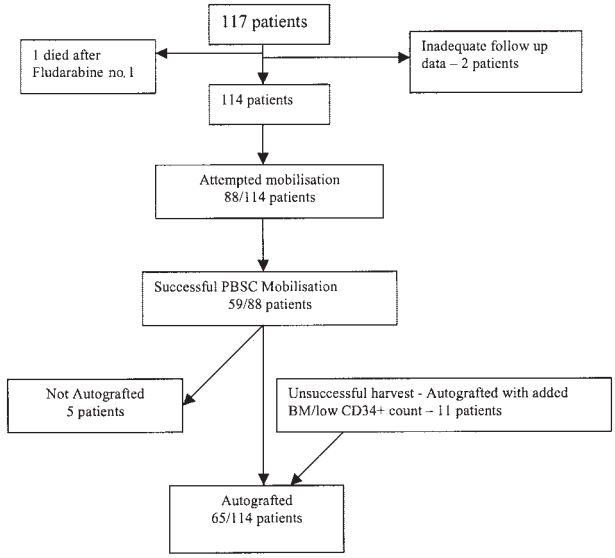

Figure 1. Flowchart outlines treatment sequence for 117 patients enrolled in the study.

Stem cell mobilization

Of 114 patients who progressed through initial and second-line therapy, mobilization was attempted in 88 (77%) and was performed using either G-CSF alone (8 patients) or in combination with cyclophosphamide (63 patients). Six patients underwent mobilization with cyclophosphamide and G-CSF followed by a further attempt at mobilization using G-CSF alone. The mobilization schedule for 11 patients was not specified. A backup marrow harvest was also performed in 16 patients in whom this was deemed necessary. Mobilization was not attempted in 26 patients (23%) for a variety of reasons, the most common being an inadequate response to therapy (Table 2).

Of the 88 patients in whom mobilization was attempted, an adequate harvest, defined as equal to or more than 2.0×10^6 $CD34^+$ cells/kg body weight was obtained in 59 (67%). Thus, 29 patients (33%) failed to mobilize peripheral blood progenitor cells. The median CD 34^+ count obtained was 2.46×10^6 cells/kg (range, 0-7.16×10^6). The 95% CI for successful mobilization was 56.2% to 76.7%. CD 34^+ cell selection was performed in 15 patients. Mobilization success was not found to correlate with sex, stage at presentation, presence of B symptoms, degree of marrow

Table 2. Reasons for not attempting mobilization in 26 patients

Reason for not attempting mobilization	No. of patients
Inadequate response/progressive CLL	15
Decision to allograft	3
Patient decision against autograft	3
Physician decision against autograft	1
Required chemotherapy for melanoma	1
Hepatitis C infection	1
Reason not specified	2

infiltration at presentation, Eastern Cooperative Oncology Group (ECOG) performance status, or response to therapy.

Autologous transplantation

Sixty-five of the 117 patients entered into this study (56%) proceeded to high-dose therapy with autologous stem cell rescue. Of these, 49 received peripheral blood progenitor cells (PBPCs) and 14 received bone marrow in addition to PBPCs; only 2 patients received bone marrow alone. The conditioning regimen used was cyclophosphamide together with TBI in 49 patients, BEAM (BCNU [bischloroethylnitrosourea], etoposide, ara-C [cytarabine], melphalan) in 11 patients, and busulphan with cyclophosphamide in a single patient. The conditioning regimen used was not specified in 4 patients. Forty-nine patients did not progress to an autograft; the reasons are summarized in Table 3.

The median time to neutrophil engraftment (neutrophil count $> 0.5 \times 10^9$/L) was 14 days (range, 10-38 days) and the median time to achieve a platelet count of more than 25×10^9/L was 13 days (range, 7-203 days). Posttransplant G-CSF was used in 31 patients (50%) for a median of 7 days (range, 2-25 days). The median in-patient stay was 19 days (range, 12-45 days). No deaths occurred during the immediate peritransplant period; however, one patient was readmitted with a fatal episode of transverse myelitis, thought to be related to therapy, 9 weeks after the autograft. Exhaustive microbiologic and virologic tests on the cerebrospinal fluid were unhelpful and permission for autopsy was refused.

Of 65 patients who had autografts, the number of patients in complete remission increased from 24 (37%) to 48 (74%) after high-dose therapy with stem cell rescue. Twenty-six of 41 patients (63%) who were not in complete remission at the time of their transplantation, achieved complete remission after high-dose therapy, 3 (7%) improved from PR to nPR, and 11 (27%) showed no change in status after autografting. One patient had no response and died due to progressive CLL after transplantation. Two further patients in complete remission before autografting, progressed to nPR following their transplantation procedure.

After a median follow-up of 3 years, 58 (89%) of the 65 patients who had an autograft are still alive, and 7 patients (11%) have died. The cause of death was progressive CLL (2 patients), therapy-related transverse myelitis (1 patient), sepsis, (1 patient), duodenal ulcer perforation with sepsis (1 patient), alveolar hemorrhage due to secondary myelodysplasia (1 patient), and unknown (1 patient). Two deaths were related to therapy, one of these occurring within the first 100 days, for an early transplant-related mortality rate of less than 2%. Forty-five of 65 patients (69%) have had no clinical progression of their disease. The remainder have progressed or had a relapse (13 of 65; 20%).

Overall and disease-free survival at 5 years of all patients entered into the study was 79.5% (CI, 69.3%-89.7%) and 48.9% (CI 37.8%-60.7%), respectively (Figure 2). The 5-year overall and disease-free survival from transplantation was 77.5% (CI, 57.2%-97.8%) and 51.5% (CI, 33.2%-69.8%), respectively (Figure 2B). After excluding primary nonresponders, patients who had autografts had a 5-year overall and disease-free survival of 88.6% (CI, 77.8%-99.4%) and 64.7% (CI, 50.4%-79.0%), respectively, whereas those who did not have autografts, had a 5-year overall and disease-free survival of 78.6% (CI, 51.3%-100%) and 28.8% (CI, 4.2%-53.4%), respectively (Figure 2C-D).

Transplant-related morbidity was not excessive. Infectious complications seen were reactivation of varicella zoster (4 patients), *Pneumocystis carinii* pneumonia (2 patients), recurrent chest infections (2 patients), and cytomegalovirus reactivation (1 patient). A significant complication noted was the occurrence of secondary myelodysplasia in 5 patients with one fatality (Table 4). Myelodysplastic syndrome (MDS) occurred only in patients who had received TBI rather than chemotherapy alone based regimens but this was not significant. The use of TBI was associated with no difference on overall survival but improved disease-free survival after autografting ($P < .05$). Other problems encountered were chronic cytopenias (2 patients), immune thrombocytopenia (2 patients), and hemolytic anemia (1 patient). We found no evidence in this study that age, sex, or stage of disease at study entry were predictors of either disease-free survival or overall survival.

Assessment of MRD

Forty-three patients, all PCR^+ prior to transplantation, had residual disease status assessed at varying time points after transplantation by framework 3 PCR (Figure 3). Data were analyzed in 6-month blocks after transplantation. All patients tested were positive at the time of transplantation, even if in complete remission. No disease was detectable by PCR at 0 to 6, 6 to 12, 12 to 18, 18 to 24, and more than 24 months in 80% (16 of 20), 62% (18 of 29), 59% (13 of 22), 60% (9 of 15), and 45% (5 of 11) of patients, respectively. Clinical complete remission prior to transplantation correlated with absence of residual disease at 6 to 12 months after transplantation with 8 of 8 patients in complete remission prior to transplantation becoming PCR^- compared to only 10 of 21 (48%) patients not in complete remission. Of 11 patients PCR^+ at 6 to 12 months after transplantation, 7 (63%) have had a clinical relapse at a median follow-up of 38 months (range, 2-49 months). In comparison, 4 of 18 patients (22%) PCR^- at this time have become PCR^+ (3 at 18 months and 1 at 36 months after transplantation) and 3 of these patients have had a clinical relapse. One further patient had a relapse at 38 months after transplantation, with no detectable disease by PCR at 30 months. Thirteen patients remain PCR^- and free of clinical relapse at a median follow-up of 33 months (range, 15-66 months).

Table 3. Reasons for not proceeding to autograft in 49 patients

Reason	Whether harvested	No.
Decision to allograft	NA*	6
Unsuccessful harvest	NA	15
Patient decision	Successful harvest	3
Hepatitis C infection	Not harvested	1
Progressive disease	NA	16
Patient decision	Not harvested	3
Melanoma/chemotherapy	Not harvested	1
Physician decision	Not harvested	1
Reason unspecified	NA	3

NA indicates not applicable.

*One patient had a successful harvest; 2 had unsuccessful harvests; and 3 were not harvested.

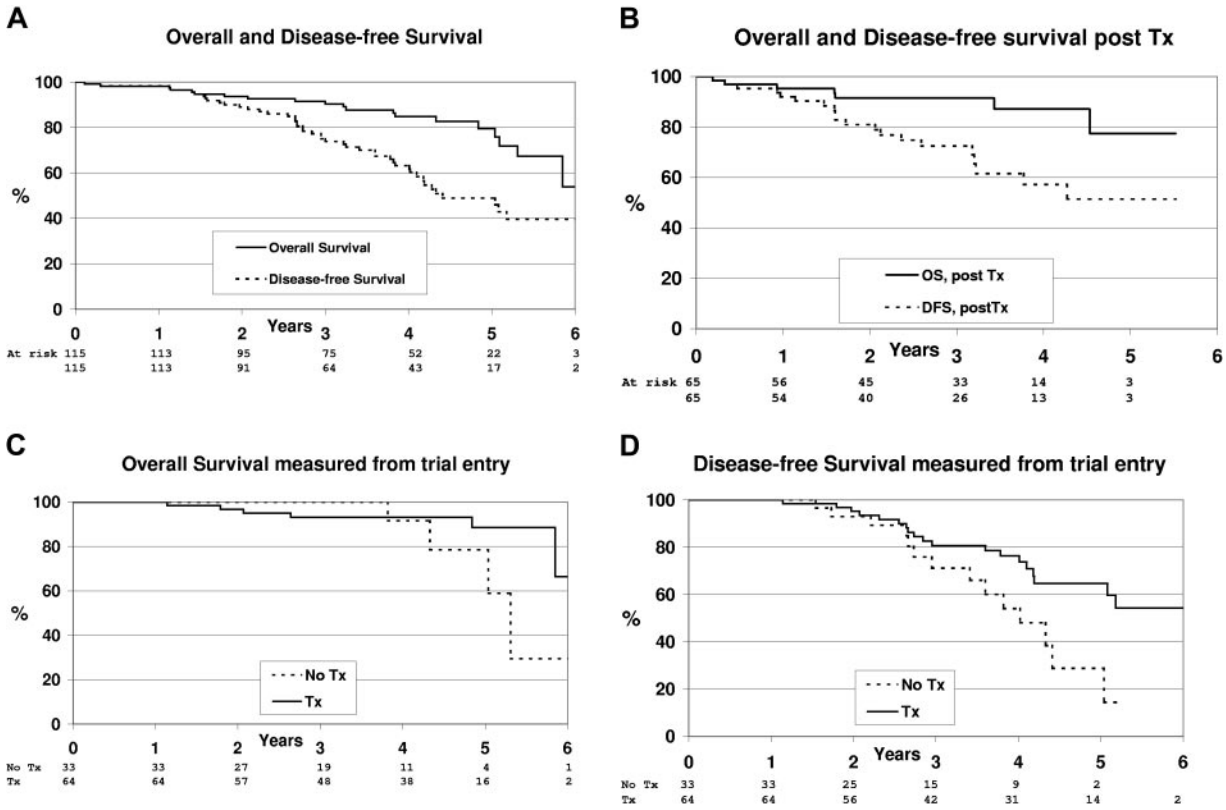

Figure 2. Overall and disease-free survival. (A) Overall and disease-free survival of all patients entered into the study. The solid line represents overall survival at 5 years, 79.5% (CI, 69.3%-89.7%). The dotted line represents disease-free survival at 5 years, 48.9% (CI, 37.8%-60.7%). (B) Overall and disease-free survival from the point of transplantation. The solid line represents overall survival of 77.5% at 5 years (CI, 57.2%-97.8%). The dotted line represents disease-free survival of 51.5% at 5 years (CI, 33.2%-69.8%). (C) Overall survival from trial entry of patients receiving an autograft and of patients not undergoing transplantation. The solid line represents overall 5-year survival (88.6%) of patients who had autografts (CI, 77.8%-99.4%). The dotted line represents overall survival of patients who did not receive an autograft, 78.6% at 5 years (CI, 51.3%-100.0%). (D) Disease-free survival measured from trial entry of patients receiving an autograft and of patients not undergoing autografting. The solid line represents disease-free survival of patients who received an autograft, 64.7% at 5 years (CI, 50.4%-79.0%). The dotted line represents disease-free survival of patients who did not receive an autograft, 28.8% at 5 years (CI, 4.2%-53.4%).

To investigate the predictive value of this molecular marker on recurrence we used a proportional hazards model with time-dependent marker variables. The presence of detectable molecular disease was highly predictive of eventual clinical disease recurrence with a hazard ratio of 9.4 (CI, 2.6-33.9; $P = .0007$).

Discussion

This pilot study is the first prospective multicenter trial addressing the use of high-dose therapy in CLL and was designed to assess the deliverability, safety, and efficacy of autologous peripheral stem cell transplantation up front, after initial treatment with fludarabine as a first-line agent. The use of nucleoside analogues such as fludarabine in the treatment of CLL has been shown to achieve overall response rates of 50% to 80% in previously untreated patients and up to 60% in patients who have had prior treatment.[7-10,12] Occasional patients may also achieve a complete immunophenotypic and molecular remission.[46] In this study of previously untreated patients, we found an overall response rate (CR + PR) of 83%, which is in keeping with other published data and confirms the role of fludarabine as an effective first-line agent for CLL. It is worth noting, however, that approximately one third of our patients required additional therapy to achieve disease control sufficient to permit progression to progenitor cell mobilization.

Several studies have confirmed the feasibility of performing autologous peripheral stem cell transplantation in patients with

Table 4. Patients developing secondary myelodysplasia after autografting

UPN	Cycles of fludarabine	Additional therapy	Time between transplantation and MDS, mo
0152	6	Cyclophosphamide	30
0176	6	Nil	28
0115	5	Nil	< 20
0109	4	COP, 2 cycles	32
0108	6	Nil	70

UPN indicates unique patient number; COP, cyclophosphamide, Oncovin, prednisone.

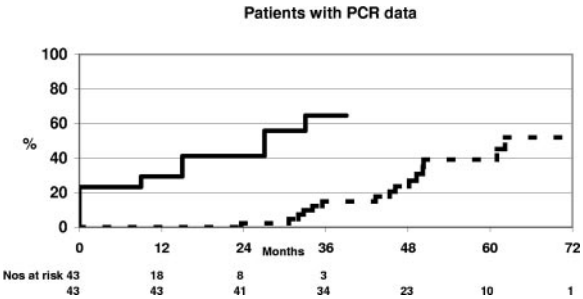

Figure 3. Relationship between molecular relapse and disease progression in patients for whom PCR data were available. The solid line represents the cumulative incidence of molecular relapse. The dotted line represents disease recurrence clinically.

CLL,[31,32,43,47] with between 64% and 75% of patients achieving a molecular remission.[38,48] These studies either examined autografting as salvage therapy or presented data only on patients who received an autograft. When used as salvage therapy approximately 40% of eligible patients can undergo transplantation, the remainder either not achieving a remission or being unable to mobilize adequate numbers of stem cells.[49] This study is the first to enroll previously untreated patients and follow them prospectively. In this setting we were able to get 65 of 115 patients (56%) to an autograft procedure.

Earlier studies examining the ability to mobilize peripheral stem cells in patients with non-Hodgkin lymphoma found that both a histologic diagnosis of small lymphocytic lymphoma/CLL and prior therapy with fludarabine were associated with a difficulty in obtaining adequate progenitor cells.[50,51] Fludarabine may also interfere with the ability to mobilize progenitor cells in acute myeloid leukemia (AML)[52] and in CLL.[53] Despite this, of 88 patients in whom we attempted stem cell mobilization, an adequate harvest was obtained in 59 (67%) though a proportion did require more than one attempt to collect adequate numbers of $CD34^+$ cells. Our figures are therefore encouraging and suggest that mobilization is possible in two thirds of patients who achieve an adequate response to initial therapy. It is possible that more effective mobilization strategies and also more intensive cytoreductive therapy to achieve better disease control prior to attempting mobilization might help yield an adequate harvest in an even greater proportion of patients.

The transplant-related mortality rate in patients receiving an autologous stem cell transplant for hematologic malignancies varies between 2% and 3.4%.[54,55] Of the 65 patients who received an autograft in our study, we had one therapy-related death within the first 100 days, for a transplant-related mortality rate of less than 2%. There has been one other death due to transplant-related MDS 19 months after transplantation. The transplant-related morbidity was also not excessive, and in the immediate posttransplant period, consisted mainly of infectious complications.

Secondary AML/MDS may be a problem. Concerns have been raised about the use of TBI containing regimens in patients with low-grade lymphoma. Age older than 40 years at the time of transplantation and receipt of a TBI-containing regimen have been shown to be predictive for developing MDS/AML in patients with non-Hodgkin lymphoma, although prior cytotoxic therapy may play a part.[56,57] Therapy-related myeloid leukemias have also been reported in patients with CLL after treatment with purine analogues, particularly in combination with alkylating agents.[58] Although the incidence of MDS in this study is worrying (5 of 65 patients; 8%), in a similar unpublished trial in Germany, with a median follow-up of over 3 years, only one case of therapy-related AML/MDS has been seen after 85 transplants in patients with CLL (P. Dreger, personal communication, April 2004). Although the numbers are too small to show a statistically significant difference between patients receiving TBI-based regimens and those not, it is interesting that posttransplant MDS occurred only in those receiving TBI. A multivariate analysis of the European Group for Blood and Marrow Transplantation database for autografting in lymphoma has already shown that TBI may be an independent risk factor for posttransplant MDS.[59]

Of the 65 patients receiving high-dose therapy in our study, 3 showed disease progression after transplantation with a further 11 patients showing no additional response to that obtained prior to their autograft. The number of patients attaining a CR after autograft increased from 37% to 74%. After excluding primary nonresponders, patients who had autografts had a 5-year overall and disease-free survival of 88.6% (CI, 77.8%, 99.4%) and 64.7% (CI, 50.4%, 79.0%), respectively, whereas those who were not autograft recipients had a 5-year overall and disease-free survival of 78.6% (CI, 51.3%, 100%) and 28.8% (CI, 4.2%, 53.4%), respectively (Figure 2C-D) These groups, however, are not directly comparable in our study because patients who underwent autografting were selected based on their response and the adequacy of the stem cell harvest.

Residual disease was undetectable by PCR in 80% of evaluable patients assessed at 6 to 12 months after transplantation, a figure comparable to other studies. Serial follow-up shows an increasing proportion of positive patients over time and it seems likely that eventually most, if not all, patients will have a molecular relapse.[60,61] Autologous transplantation for CLL is known to have a high rate of clinical relapse with a projected rate of 56% at 3 years,[39,62] and in our study a strong correlation was found between relapse and the presence of detectable residual disease between 6 and 12 months after autografting. Patients with a sustained molecular remission were significantly less likely to have a relapse clinically.

It would have been helpful to have had cytogenetic analysis for the patients in this study but at the time of trial design this was not easily available in the United Kingdom. It is likely that cytogenetic abnormalities, particularly an unmutated immunoglobulin heavy-chain gene (IgV_H) will help in risk stratifying patients with CLL more accurately to tailor therapeutic strategies, depending on risk group. Patients with unmutated IgV_H genes appeared to benefit from high-dose therapy in a risk-matched analysis,[37] and it is likely that further refinements in risk stratification will help select patients who will benefit most from this approach.

We conclude that autologous stem cell transplantation is feasible and relatively safe as a treatment modality in suitable patients with CLL. There is also a need to compare autologous stem cell transplantation with an expectant approach in young patients with high-risk CLL who respond well to conventional or second-line treatment, and the new European Intergroup/Medical Research Council Trial (MRC CLL 5 Trial) aims to address this issue.

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Appendix

Participating centers (transplantation centers are marked with an asterisk) were: Addenbrookes Hospital* (Dr Marcus), Altnagelvin Area Hospital (Dr Ryan), Basildon Hospital (Dr Watts), Birmingham Heartlands Hospital* (Dr Fegan, Dr Johnson, Dr Milligan), Bishop Auckland Hospital (Dr Galloway), Burton Hospital (Dr Smith), Christchurch Hospital, New Zealand* (Dr Brozovic, Dr Hart, Dr Patton, Dr Spearing), City Hospital Birmingham (Dr Bareford), Countess of Chester Hospital (Dr Rhodes), Darlington Memorial Hospital (Dr. Williamson), Derby Royal Infirmary (Dr Mitchell), Good Hope Hospital Birmingham (Dr Hamilton, Dr Tucker), Law Hospital (Dr Helenglass), Leeds General Infirmary* (Dr Child, Dr Hillmen, Prof Morgan), Leicester Royal Infirmary* (Dr Wood), Monklands Hospital (Dr. Murphy), Mount Vernon Hospital (Dr McMillan), Neville Hall Hospital (Dr Habboush), North Staffordshire Hospital (Dr Chasty), Nottingham City Hospital* (Dr Haynes, Prof Russell), Pinderfields Hospital (Dr Hillman), Poole General Hospital (Dr Bell), Royal Gwent Hospital (Dr Moffatt), Royal London Hospital* (Dr Cavenagh, Dr Kelsey), Royal Marsden Hospital* (Prof Catovsky, Dr Cunningham), Royal South Hants Hospital* (Dr Duncombe, Dr Smith), Royal Surrey Hospital (Dr Robbins), Southend Hospital (Dr Eden, Dr Mills), Staffordshire General Hospital (Dr Revell), The Group Practice Health Centre (Dr Dickie), West Cumberland Hospital (Dr West).

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