

ORIGINAL ARTICLE

Autologous stem cell transplantation in patients with chronic lymphocytic leukaemia: the Finnish experience

E Jantunen¹, M Itälä², T Siitonen³, E Juvonen⁴, E Koivunen⁵, P Koistinen³, L Volin⁴, K Remes² and T Nousiainen¹

¹Department of Medicine, Kuopio University Hospital, Kuopio, Finland; ²Department of Medicine, Turku University Central Hospital, Turku, Finland; ³Department of Medicine, Oulu University Hospital, Oulu, Finland; ⁴Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland and ⁵Department of Medicine, Tampere University Hospital, Tampere, Finland

Although autologous stem cell transplantation (ASCT) has gained some popularity as a treatment option in patients with chronic lymphocytic leukaemia (CLL), limited multicentre data are available on the feasibility and efficacy of this approach. Between January 1995 and June 2005, 72 patients with CLL received ASCT in five Finnish centres. There were 45 men and 27 women with a median age of 57 years (38–69). The median time from diagnosis to ASCT was 32 months (6–181) and the median number of prior regimens 1 (1–4). All patients received blood stem cell grafts and CD34⁺ selection had been performed in 44 patients (61%). The most common high-dose regimen was a total body irradiation plus cyclophosphamide (38 patients, 53%). No early treatment-related deaths were observed. With a median follow-up of 28 months from ASCT, a relapse or progression has been observed in 27 patients (37%). The projected progression-free survival is 48 months (confidence interval (CI) 30–66). The projected median overall survival is 95 months (CI 74–101) from ASCT and is not influenced by graft selection or conditioning regimen used. Autologous stem cell transplantation is a feasible treatment option for CLL. Randomized trials against alternative treatments are needed to assess the impact of ASCT on the clinical course of CLL.

Bone Marrow Transplantation (2006) 37, 1093–1098.
doi:10.1038/sj.bmt.1705375; published online 8 May 2006

Keywords: autologous stem cell transplantation; chronic lymphocytic leukaemia; feasibility; outcome

Introduction

Chronic lymphocytic leukaemia (CLL) is a chronic lymphoproliferative disorder characterized by proliferation of clonal B lymphocytes in peripheral blood, lymph nodes, bone marrow and spleen. The median age of the patients with CLL is more than 65 years at diagnosis. In many patients, CLL is characterized by indolent clinical course and no need for any treatment for years. On the other hand, patients with Binet stage C have a median survival of only a few years. In addition to clinical stage, also cytogenetic features¹ and mutational status of immunoglobulin genes² have been shown to be of prognostic significance.

Although CLL is chemosensitive to alkylating agents and nucleoside analogues, there are no data indicating that this disease can be cured by conventional chemotherapy. Although fludarabine (FLU) alone³ or combined with cyclophosphamide⁴ (CY) gives high response rates in untreated patients, the disease invariably recurs and chemoresistance and infectious problems emerge in the majority of patients making further therapy difficult and responses less likely. Combinations of antibodies like alemtuzumab^{5–7} and rituximab^{8,9} with chemotherapy have produced promising response rates and long-term data are awaited with interest.

As a significant number of patients with CLL are relatively young and many of them have poor prognosis, more intensive approaches including autologous and allogeneic stem cell transplantations (SCT) have been applied.^{10–13} Most reports on the use of ASCT in patients with CLL are from single centres^{14–17} with the notable exception of a prospective multicentre MRC trial.¹⁸ We have evaluated in a nation-wide multicentre setting, the feasibility and efficacy of ASCT in patients with CLL.

Patients and methods

Patients

Altogether 72 patients with CLL received ASCT in five Finnish university hospitals between January 1995 and

Correspondence: Dr E Jantunen, Department of Medicine, Kuopio University Hospital, PO Box 1777, 70211 Kuopio, Finland.
E-mail: esa.jantunen@kuh.fi
Received 24 January 2006; revised 23 March 2006; accepted 25 March 2006; published online 8 May 2006

Table 1 Major characteristics of 72 patients with CLL who received ASCT in Finland 1995–2005

	No. (%)
<i>Gender</i>	
Female	27 (38)
Male	45 (62)
<i>Age at ASCT, median (range)</i>	
> 60 years	57 (38–69) 29 (40)
<i>No. of previous treatment lines before ASCT</i>	
1	41 (57)
2	22 (31)
> 2	9 (12)
<i>Previous therapy</i>	
Fludarabine	54 (75)
Fludarabine–cyclophosphamide	12 (17)
CHOP-like	23 (32)
Chlorambucil	8 (11)
Monoclonal antibodies	9 (13)
<i>Disease status at ASCT</i>	
CR	22 (30)
PR	46 (64)
NR	4 (6)
<i>Time from diagnosis to ASCT, months</i>	
Median (range)	32 (6–181)

Abbreviations: ASCT = autologous stem cell transplantation; CHOP = cyclophosphamide, doxorubicin, vincristine, prednisolone; CR = complete remission; PR = partial remission; NR = non-responder.

June 2005. Initially 35 patients (49%) were diagnosed to have Binet stage A, 29 patients (40%) Binet B and eight patients (11%) were in stage C. Cytogenetic study was initially performed in 30 patients (42%): no cytogenetic changes were observed in 10 patients, whereas a pathologic karyotype was observed in 20 patients (+ 12 five patients, 11q- four patients, 13q- four patients, 13q14 four patients, 17p- three patients). The indications to proceed to ASCT varied during time but in early years, ASCT was mainly used for second- or third-line treatment, whereas more recently younger patients were also considered for ASCT in first complete remission (CR) or partial remission (PR) after FLU-based induction therapy.

The number of patients transplanted per centre during the study period varied from five to 29. The annual number of transplants was highest in 2001 and 2003 (13 patients in both years). The major characteristics of transplanted patients are summarized in Table 1.

Progenitor cell mobilization and collection

All patients had received debulking chemotherapy before progenitor cell mobilization. The median number of chemotherapy cycles (excluding chlorambucil) was five (2–19). Altogether 66 patients (92%) had received FLU before progenitor cell mobilization; the median number of prior cycles with FLU was four (1–12). Just before mobilization, the median percentage of lymphocytosis in marrow aspirate was 20%; 22 patients (30%) had $\geq 30\%$ lymphocytes in marrow examination.

Sixty-one patients (85%) received intermediate-dose CY (4 g/m²) plus granulocyte colony-stimulating factor (G-CSF) 5–10 $\mu\text{g}/\text{kg}/\text{day}$ for progenitor cell mobilization. Low-dose CY (2 g/m²) plus G-CSF was used in additional nine patients (13%). Two patients were mobilized with G-CSF 10 $\mu\text{g}/\text{kg}/\text{day}$ alone.

High-dose therapy protocols

Total body irradiation plus cyclophosphamide (TBI-CY) was the most commonly applied high-dose regimen (38 patients, 53%) followed by BEAC ($N=27$) and BEAM ($N=6$). All patients received blood progenitor cell grafts. CD34⁺ selection had been performed in 44 patients (61%). Altogether 61 patients (85%) received G-CSF after the progenitor cell infusion

Response criteria

The response to conventional dose therapy as well as to high-dose therapy (HDT) was evaluated according to the National Cancer Institute criteria¹⁹ and was based in addition to blood counts and differentials also on the evaluation bone marrow aspirate and computed tomography. For more precise response evaluation, also more sophisticated methods including fluorescent *in situ* hybridization, flow cytometry and polymerase chain reaction (PCR) were used during the study period in some centres. As these methods were not used in all patients, these results are not included in this report.

Complete remission was defined as absence of lymphocytosis ($< 4 \times 10^9/\text{l}$) in peripheral blood and marrow aspirate ($< 30\%$) with haemoglobin $> 110 \text{ g/l}$, neutrophils $> 1.5 \times 10^9/\text{l}$ and platelets $> 100 \times 10^9/\text{l}$. Also normal spleen size and absence of lymphadenopathy were required. Partial remission was defined as at least 50% decrease in lymphadenopathy or splenomegaly, improvement of blood counts by at least 50% or haemoglobin $> 110 \text{ g/l}$, neutrophils $> 1.5 \times 10^9/\text{l}$, platelets $> 100 \times 10^9/\text{l}$; in addition to a decrease of $> 50\%$ in the absolute lymphocyte count. All other outcomes were defined as non-response.

Definition of relapse or progression after autologous stem cell transplantation

In patients who attained a CR after ASCT, the relapse was defined as emergence of lymphocytosis with or without lymphocyte infiltration in marrow specimen. Also lymphadenopathy with a histologic verification of lymphocytic lymphoma/diffuse large B-cell lymphoma was considered a relapse. In patients who achieved only PR after ASCT, a progression was defined as 50% increase in peripheral blood lymphocytosis and/or lymphadenopathy.

Statistical analysis

All analyses were performed by using SPSS 11.0 for Windows (SPSS, Chicago, IL, USA). For univariate analysis of factors predicting outcome, the means were compared by using Mann–Whitney U-test. The χ^2 test was used to compare nominal data. Progression-free survival (PFS) and overall survival (OS) were computed from the date of ASCT with Kaplan–Meier method.

A relapse or progression was regarded as a competing risk for treatment-related mortality (TRM).

Results

Progenitor cell mobilization and aphaeresis

Median peak B-CD34⁺ count measured after the mobilization was $46 \times 10^6/l$ (8–244). With the median of two aphaeresis (1–4), a median of $4.6 \times 10^6/kg$ (0.75–18.3) CD34⁺ cells were collected. At least $2 \times 10^6/kg$ CD34⁺ cells were collected after the first mobilization attempt in 66 patients (92%).

Six patients who experienced a mobilization failure were all subsequently successfully re-mobilized.

Engraftment and toxicity after high-dose therapy

All patients engrafted after HDT. The medians to reach neutrophils $>0.5 \times 10^9/l$ and platelets $>20 \times 10^9/l$ were 11 (8–17) and 12 (8–31) days, respectively.

Sixty-four patients (89%) experienced neutropenic fever (>38 C) after ASCT. Blood cultures were positive in 29 patients (40%). The most common blood culture findings included *Staphylococcus epidermidis* ($N=11$), *Streptococcus viridans* ($N=6$) and *Escherichia coli* ($N=5$). Radiologically verified pneumonia was observed in seven patients (10%). No early (<100 days) treatment-related deaths were observed and none of the patients needed intensive care admission during ASCT. The median number of in-hospital days during HDT was 24 days (17–50); 13 patients (18%) needed hospital care for at least 4 weeks.

Outcome after autologous stem cell transplantation

All patients are evaluable for response after ASCT. Altogether sixty-two patients (86%) achieved or maintained CR after ASCT, eight patients (11%) were in PR and two patients were non-responders. During the follow-up, myelodysplastic syndrome (MDS) has been observed in two patients (2.8%) at 10 and 19 months post-ASCT, respectively. No cases of acute myeloid leukaemia (AML) have been observed.

The projected OS for all patients at 5 years was 80% (Figure 1) and the median survival, 95 months (95% confidence interval (CI) 74–101). Until November 2005, 16 patients (22%) have died. Ten patients (14%) died due to progression of CLL, whereas six patients (8%) died from non-relapse reasons 101–2057 days from ASCT (infection three, glioma one, MDS one, cardiac failure one). Age at transplant (<60 vs ≥ 60 years), gender, conditioning regimen used (TBI-based vs other) or graft selection were not associated with OS in univariate analysis. Neither remission status at transplant (CR vs other status) nor the number of previous treatment lines (1 vs >1) had significant impact on OS.

With a median follow-up of 28 months (5–120+) for all patients, a relapse or progression has been observed in 27 patients (37%). The projected median PFS was 48 months (95% CI 30–66) from ASCT (Figure 2). Age, gender, conditioning regimen used or graft selection had no significant impact on PFS in univariate analysis. Remission

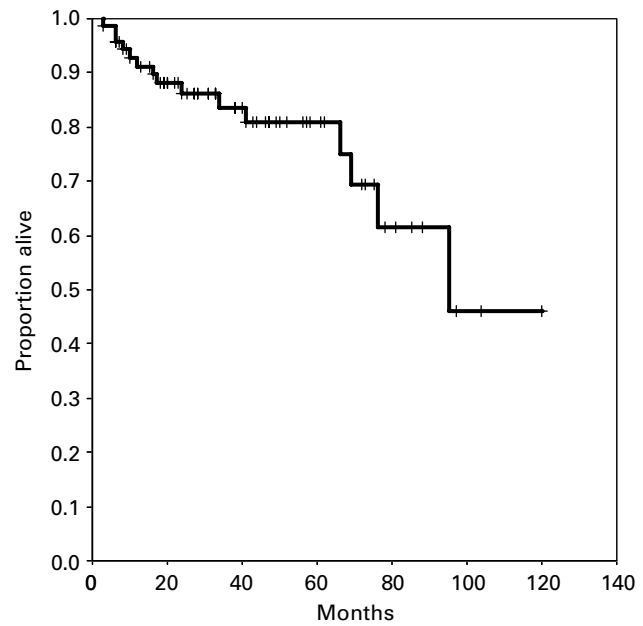


Figure 1 Overall survival after autologous stem cell transplantation in 72 patients with chronic lymphocytic leukaemia.

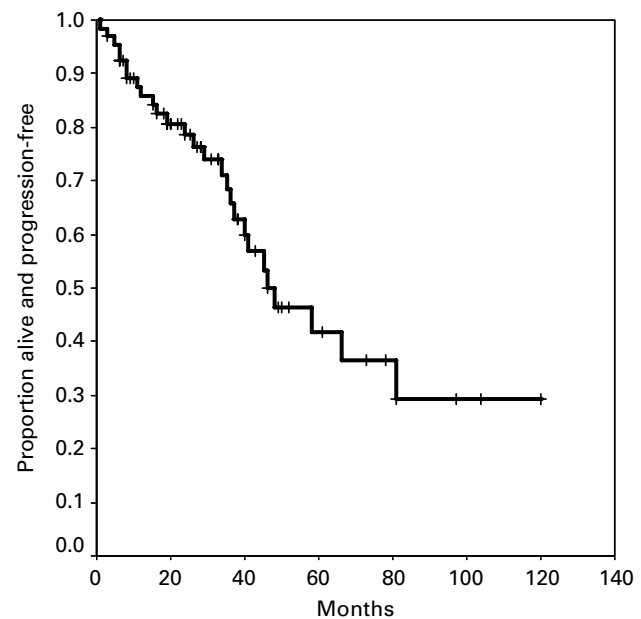


Figure 2 Progression-free survival after autologous stem cell transplantation in 72 patients with chronic lymphocytic leukaemia.

status at transplant had no significant impact on PFS either (Figure 3). Patients who received ASCT after first-line treatment tended to have longer PFS than patients transplanted later during the disease course (58 vs 41 months, $P=0.09$). The median time from ASCT to relapse or progression was 26 months (1–81). A histological transformation was observed at relapse in four patients.

Until now, 20 patients have been treated for relapse or progression after ASCT (Table 2). Five patients (25%)

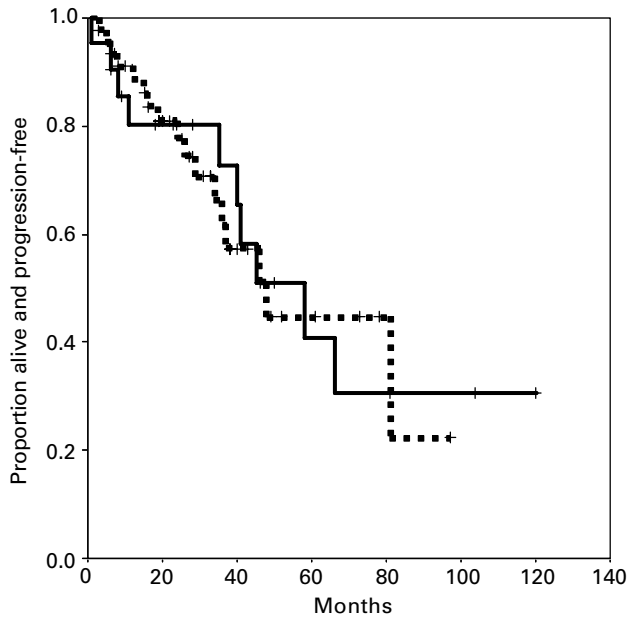


Figure 3 Progression-free survival after autologous stem cell transplantation in patients transplanted in complete remission (solid line) and those transplanted in other disease status (dotted line). $P =$ not significant.

Table 2 Treatment of progressive disease after ASCT in 20 patients with CLL

Regimen	No. of patients	Response to salvage therapy		
		CR	PR	NR
FC	4		2	2
F	1	1		
R-FC	2	1	1	
Alemtuzumab	4	2	2	
Rituximab	1			1
CHOP-like	2		1	1
ESHAP	1			1
Cladribine + rituximab	1		1	
Chlorambucil	2	1		1
Cyclophosphamide	2			2

Abbreviations: CR = complete remission; PR = partial remission; NR = non-response; CHOP = cyclophosphamide, doxorubicin, vincristine, prednisolone; ESHAP = etoposide-methylprednisolone-cytosine arabinoside-platinum; F = fludarabine; FC = fludarabine-cyclophosphamide; R = rituximab.

achieved a CR after salvage therapy and seven patients, (35%) a PR with an objective response rate of 60%. Two patients underwent allografting with reduced intensity conditioning and two additional patients are now considered for allografting. With a median follow-up of 32 months (0–50+) from relapse of progression, 13 patients (48%) are still alive.

Discussion

The main findings of this multicentre nation-wide analysis were low treatment-related mortality, despite the fact that a

significant percentage of the patients were ≥ 60 years at transplant, a high percentage of remissions and reasonable OS. However, there seems to be no plateau in PFS suggesting curative potential of this approach.

Previous studies of ASCT in CLL are mainly single centre based.^{10,14–17} The largest experience published to date comes from Boston and includes 137 patients treated with TBI-CY plus purged BM autograft. The main findings were a 100-days TRM of 4% and no evidence of plateau in PFS.¹⁷ The first prospective multicentre trial was performed by MRC and published recently.¹⁸ They concluded that the procedure (TBI-CY + blood stem cell graft) was safe (100 d mortality <2%) and induced molecular remissions in a significant proportion of patients. A rather high incidence of MDS or AML (8%) was, however, a concern. We have observed only two proven cases of MDS until now and no cases of secondary AML. Of note, no early toxic deaths were observed in our nation-wide series.

Previous studies have suggested that a significant proportion of patients with CLL may be difficult to mobilize.^{20–22} We requested mobilization data only for patients who actually were transplanted. Therefore, our success rate of progenitor cell mobilization is likely to be an overestimation. However, in the majority of the patients grafts large enough to proceed to CD34⁺ selection were collected. Selection of the graft may increase the risk of infectious complications.²³ Our retrospective analysis suggests that graft selection does not affect either PFS or OS. The decisive answer to the potential role of graft selection will be reached only by a randomized study utilizing surrogate markers of minimal residual disease like flow cytometry or PCR.

Indications to proceed to ASCT in this retrospective series varied. This is mainly due to the fact that there are no randomized studies evaluating the role of HDT in patients with CLL. Recently, new prognostic markers have been observed. These include in addition to clinical stage and lymphocyte doubling time also mutational status,^{16,24,25} cytogenetics^{1,26} and most recently microRNA signatures.²⁷ Although these markers are useful, at present there are no prospective studies evaluating the efficacy of various treatment options in patients regarded as having poor prognosis based on these markers.

The projected median PFS was 4 years in this series. Interestingly, a recent study using FLU + CY in previously untreated patients found a median PFS of also 4 years.⁴ These results are not comparable as >40% of our patients had received 2–4 lines of therapy before ASCT. It might be possible to improve the current results of ASCT further by performing prospective residual disease monitoring and using additional immunotherapy after ASCT in patients with residual disease. Given the availability of better prognostic markers and advances in the management of CLL including monoclonal antibodies like alemtuzumab^{5–7} and rituximab,^{8,9,28} a randomized trial of ASCT against modern chemoimmunotherapy regimens seems worthwhile.

Although many patients with CLL enjoy prolonged PFS and OS after ASCT, the procedure does not seem to be curative. Therefore, some centres have moved away from ASCT to reduced-intensity conditioned (RIC) allografts.¹⁷ Graft-versus-leukaemia effect of allogeneic SCT has been

clearly shown in patients with CLL.^{17,29–33} Reduced-intensity conditioned allografting might be the preferred approach over autografting in younger, generally fit patients with suitable donor and poor prognostic features. However, not all patients with CLL are young, have suitable donors or are fit and some of these patients might be considered for autografting.

Limited data are available on the outcome of CLL patients who progress after ASCT. Our analysis showed that a high number of responses can be obtained in relapsed patients in analogy with patients having follicular lymphoma.³⁴ This gives opportunity at least in some patients to consider RIC allografting at this point. In many patients, the relapse had a rather indolent course, but also some cases presenting with histologic transformation and poor prognosis were observed.

To conclude, this retrospective multicentre survey showed a low treatment-related mortality and a low risk of MDS. The outcome was in general promising although no plateau in survival curves was observed. Although ASCT seems not to be curative in patients with CLL, it is still worth considering in selected patients. Randomized trials are needed to evaluate ASCT with current non-transplant regimens and also allografting.

Acknowledgements

The study was supported by a grant from the Blood Disease Research Foundation in Finland.

References

- 1 Döhner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L *et al*. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med* 2000; **343**: 1910–1916.
- 2 Hamblin TJ, Davis Z, Gardiner A, Oschier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood* 1999; **94**: 1848–1854.
- 3 Rai KR, Peterson BL, Appelbaum FR, Kolitz J, Elias L, Shepherd L *et al*. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. *N Engl J Med* 2000; **343**: 1750–1757.
- 4 Eichhorst BF, Busch R, Hopfinger G, Pasold R, Hensel M, Steinbrecher C *et al*. Fludarabine plus cyclophosphamide versus fludarabine alone in first line therapy in younger patients with chronic lymphocytic leukemia. *Blood* 2006; **107**: 885–891.
- 5 Lundin J, Kimby E, Björkholm M, Broliden PA, Celsing F, Hjalmar V *et al*. Phase II trial of subcutaneous anti-CD52 monoclonal antibody alemtuzumab (Campath-1H) as first-line treatment for patients with B-cell chronic lymphocytic leukemia (B-CLL). *Blood* 2002; **100**: 768–773.
- 6 Keating MJ, Flinn I, Jain V, Binet JL, Hillmen P, Byrd J *et al*. Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. *Blood* 2002; **99**: 3554–3561.
- 7 Elter T, Borchmann P, Schulz H, Reiser M, Trelle S, Schnell R *et al*. Fludarabine combination with alemtuzumab is effective and feasible in patients with relapsed or refractory B-cell chronic lymphocytic leukemia: results of a phase II trial. *J Clin Oncol* 2005; **23**: 7024–7031.

- 8 Keating MJ, O'Brien S, Albitar M, Lerner S, Plunkett W, Giles F *et al*. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. *J Clin Oncol* 2005; **23**: 4009–4012.
- 9 Wierda W, O'Brien S, Wen S, Faderl S, Garcia-Manero G, Thomas D *et al*. Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab for relapsed and refractory chronic lymphocytic leukemia. *J Clin Oncol* 2005; **23**: 4070–4078.
- 10 Dreger P, von Neuhoff N, Kuse R, Sonnen R, Glass B, Uharek L *et al*. Early stem cell transplantation for chronic lymphocytic leukaemia: a chance of cure? *Br J Cancer* 1998; **77**: 2201–2207.
- 11 Dreger P, Montserrat E. Autologous and allogeneic stem cell transplantation for chronic lymphocytic leukemia. *Leukemia* 2002; **16**: 985–992.
- 12 Jabbour E, Keating MJ, Champlin RE, Khouri IF. Stem cell transplantation for chronic lymphocytic leukaemia: should not more patients get a transplant. *Bone Marrow Transplant* 2004; **34**: 289–297.
- 13 Paneesha S, Milligan DW. Stem cell transplantation for chronic lymphocytic leukaemia. *Br J Haematol* 2005; **128**: 145–152.
- 14 Rabinowe SN, Soiffer RJ, Gribben J, Daley H, Freedman AS, Daley J *et al*. Autologous and allogeneic bone marrow transplantation for poor prognosis patients with B-cell chronic lymphocytic leukemia. *Blood* 1993; **82**: 1366–1376.
- 15 Khouri IF, Keating MJ, Vriesendorp HM, Reading CL, Przepiorka D, Huh YO *et al*. Autologous and allogeneic bone marrow transplantation for chronic lymphocytic leukemia: preliminary results. *J Clin Oncol* 1994; **12**: 748–758.
- 16 Ritgen M, Lange A, Stilgenbauer S, Dohner H, Bretscher C, Bosse H *et al*. Unmutated immunoglobulin variable heavy-chain gene status remains an adverse prognostic factor after autologous stem cell transplantation for chronic lymphocytic leukemia. *Blood* 2003; **101**: 2049–2053.
- 17 Gribben JG, Zahrieh D, Stephans K, Bartlett-Pandite L, Alyea EP, Fisher DC *et al*. Autologous and allogeneic stem cell transplantation for poor risk chronic lymphocytic leukemia. *Blood* 2005; **106**: 4389–4396.
- 18 Milligan DW, Fernandes S, Dasgupta R, Davies FE, Matutes E, Fegan CD *et al*. Results of MRC pilot study show autografting for younger patients with chronic lymphocytic leukaemia is safe and achieves a high percentage of molecular responses. *Blood* 2005; **105**: 397–404.
- 19 Cheson BD, Bennett JM, Grever M, Kay N, Keating MJ, O'Brien S *et al*. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood* 1996; **87**: 4990–4997.
- 20 Itälä M, Pelliniemi TT, Rajamäki A, Remes K. Autologous stem cell transplantation in B-CLL: response to chemotherapy prior to mobilization predicts the stem cell yield. *Bone Marrow Transplant* 1997; **19**: 647–651.
- 21 Michallet M, Thiebaut A, Dreger P, Remes K, Milpied N, Santini G *et al*. Peripheral blood stem cell (PBSC) mobilization and transplantation after fludarabine therapy in chronic lymphocytic leukaemia (CLL): a report of the European Blood and Marrow Transplantation (EBMT) CLL subcommittee on behalf of the EBMT Chronic Leukaemia Working Party (CLWP). *Br J Haematol* 2000; **108**: 595–601.
- 22 Tournhillac O, Cazin B, Lepretre S, Divine M, Maloum K, Delmer A *et al*. Impact of frontline fludarabine and cyclophosphamide combined treatment on peripheral blood stem mobilization in B-cell chronic lymphocytic leukaemia. *Blood* 2004; **103**: 363–365.

- 23 Altes A, Sierra J, Esteve J, Martin-Henan G, Marin P, Sureda A *et al*. CD34⁺-enriched-CD19⁺-depleted autologous peripheral blood stem cell transplantation for chronic lymphoproliferative disorders: high purging efficiency but increased risk of severe infections. *Exp Hematol* 2002; **30**: 824–830.
- 24 Dreger P, Stilgenbauer S, Benner A, Ritgen M, Krober A, Kneba M *et al*. The prognostic impact of autologous stem cell transplantation in patients with chronic lymphocytic leukaemia: a risk-matched analysis on the VH gene mutational status. *Blood* 2004; **103**: 2850–2858.
- 25 Rassenti LZ, Huynh L, Toy TL, Chen L, Keating MJ, Gribben JG *et al*. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. *N Engl J Med* 2004; **351**: 893–901.
- 26 Byrd JC, Gribben JG, Peterson BL, Grever MR, Lozanski G, Lucas DM *et al*. Select high-risk genetic features predict earlier progression following chemoimmunotherapy with fludarabine and rituximab in chronic lymphocytic leukemia: justification for risk-adopted therapy. *J Clin Oncol* 2006; **24**: 437–443.
- 27 Calin GA, Ferracin M, Cimmino A, di Leva G, Shimizu M, Wojcik SE *et al*. A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med* 2005; **353**: 1768–1771.
- 28 Del Poeta G, Del Principe MI, Irno Consalvo MA, Maurillo L, Buccisano F, Venditti A *et al*. The addition of rituximab to fludarabine improves clinical outcome in untreated patients with ZAP-70-negative chronic lymphocytic leukemia. *Cancer* 2005; **104**: 2743–2752.
- 29 Dreger P, Brand R, Hansz J, Milligan D, Corradini P, Finke J *et al*. Treatment-related mortality and graft-versus-leukemia activity of allogeneic stem cell transplantation for chronic lymphocytic leukemia using intensity-reduced conditioning. *Leukemia* 2003; **17**: 841–848.
- 30 Schetelig J, Thiede C, Bornhauser M, Schwerdtfeger R, Kiehl M, Beyer J *et al*. Evidence of a graft-versus-leukemia effect in chronic lymphocytic leukemia after reduced-intensity conditioning and allogeneic stem cell transplantation: the Cooperative German Transplant Study Group. *J Clin Oncol* 2003; **21**: 2747–2753.
- 31 Ritgen M, Stilgenbauer S, von Neuhoff N, Humpe A, Gruggemann M, Pott C *et al*. Graft-versus-leukemia activity may overcome therapeutic resistance of chronic lymphocytic leukaemia with unmutated immunoglobulin variable heavy-chain gene status: implication of minimal residual disease measurement with quantitative PCR. *Blood* 2004; **104**: 2600–2602.
- 32 Moreno C, Villamor N, Colomer D, Esteve J, Martino R, Nomdedeu J *et al*. Allogeneic stem-cell transplantation may overcome the adverse prognosis of unmutated VH gene in patients with chronic lymphocytic leukaemia. *J Clin Oncol* 2005; **23**: 3433–3438.
- 33 Sorror ML, Maris MB, Sandmaier BM, Storer BE, Stuart MJ, Hagenbart U *et al*. Hematopoietic cell transplantation after nonmyeloablative conditioning for advanced chronic lymphocytic leukaemia. *J Clin Oncol* 2005; **23**: 3819–3829.
- 34 Kuittinen T, Wiklund T, Remes K, Elonen E, Lehtinen T, Kuittinen O *et al*. Outcome of progressive disease after autologous stem cell transplantation in patients with non-Hodgkin's lymphoma: a nation-wide survey. *Eur J Haematol* 2005; **75**: 199–205.