TRANSPLANTATION

TP53, *SF3B1*, and *NOTCH1* mutations and outcome of allotransplantation for chronic lymphocytic leukemia: six-year follow-up of the GCLLSG CLL3X trial

Peter Dreger,[1] Andrea Schnaiter,[2] Thorsten Zenz,[1-3] Sebastian Böttcher,[4] Marianna Rossi,[5] Peter Paschka,[2] Andreas Bühler,[2] Sascha Dietrich,[1] Raymonde Busch,[6] Matthias Ritgen,[4] Donald Bunjes,[2] Matthias Zeis,[7] Michael Stadler,[8] Lutz Uharek,[9] Christof Scheid,[10] Ute Hegenbart,[1] Michael Hallek,[10] Michael Kneba,[4] Norbert Schmitz,[7] Hartmut Döhner,[2] and Stephan Stilgenbauer[2]

[1]Department of Medicine V, University of Heidelberg, Heidelberg, Germany; [2]Department of Medicine III, University of Ulm, Ulm, Germany; [3]Department of Translational Oncology, National Center for Tumor Diseases and German Cancer Research Center, Heidelberg, Germany; [4]Department of Medicine II, University Hospital of Schleswig-Holstein, Kiel, Germany; [5]Division of Hematology, University of Pavia, Pavia, Italy; [6]Institute of Medical Statistics and Epidemiology, Technical University Munich, Munich, Germany; [7]Department of Hematology and Stem Cell Transplantation, Asklepios Klinik St. Georg, Hamburg, Germany; [8]Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hanover, Germany; [9]Department of Medicine for Hematology, Oncology and Tumor Immunology, Charite Campus Virchow-Klinikum, Universitätsmedizin Berlin, Berlin, Germany; and [10]Department of Medicine I, Center for Integrated Oncology Cologne Bonn, University of Cologne, Cologne, Germany

Key Points

- This trial update shows that allotransplantation can provide long-term minimal residual disease–negative disease control in poor-risk chronic lymphocytic leukemia.
- Six-year survival is close to 60% and is independent of the presence of *TP53*, *SF3B1*, and *NOTCH1* mutations in the tumor clone.

The purpose of this analysis was to provide 6-year follow-up of the CLL3X trial, which studied reduced-intensity allogeneic hematopoietic stem cell transplantation (HSCT) in patients with poor-risk chronic lymphocytic leukemia (CLL), and to investigate the effect of *TP53, SF3B1*, and *NOTCH1* mutations on HSCT outcome. For 90 allografted patients, 6-year overall survival (OS) was 58% and 6-year event-free survival (EFS) was 38%. *TP53*, *SF3B1*, and *NOTCH1* mutations were found in 30%, 26%, and 14% of the trial population, respectively. By univariate and multivariate analyses, the mutational status of the *TP53*, *SF3B1*, and *NOTCH1* genes had no significant effect on OS and EFS. Studies of minimal residual disease confirmed durability of CLL eradication in mutated patients. We conclude that HSCT can provide long-term disease control in patients with poor-risk CLL independent of the presence of *TP53, SF3B1*, and *NOTCH1* mutations. The trial has been registered at the US National Cancer Institute as #EU-20554, NCT00281983. (*Blood*. 2013;121(16):3284-3288)

Introduction

There is ample evidence that poor-risk chronic lymphocytic leukemia (CLL), as defined by fludarabine refractoriness or the presence of deletion 17p (17p-), can be successfully treated by allogeneic hematopoietic stem cell transplantation (HSCT).[1-7] It is unknown, however, whether disease control provided by HSCT is durable in the long term and whether HSCT can also overcome the treatment resistance associated with genetic factors, such as *TP53* mutations, seen under conventional fludarabine combination therapy.[8-10] Therefore, the purpose of this analysis was to provide 6-year follow-up of the German CLL Study Group (GCLLSG) CLL3X trial, which aimed to evaluate reduced-intensity HSCT in patients with poor-risk CLL, and to compare the effect of *TP53* mutations with that of the newly identified mutations of the *SF3B1* and *NOTCH1* genes, which in some studies have been associated with resistance to conventional treatment.[11-16]

Study design

The protocol including the informed consent form was approved by all responsible institutional review boards. Patients gave written informed consent using study-specific forms, in accordance with the Declaration of Helsinki.

The CLL3X trial has been described previously.[6] In brief, CLL3X included 100 patients with a median age of 53 years (27 to 65 years), of whom 69 patients met at least 1 of the EBMT CLL transplant criteria[17] and 31 patients had failed a previous autograft or fulfilled other poor-risk criteria as defined in the protocol.[6] Ninety-six percent of the patients had an unfavorable *IGHV* mutational status. Ninety patients were allografted with blood stem cells from related (40%) or unrelated (60%) donors after fludarabine-cyclophosphamide-based conditioning. A history of documented fludarabine resistance was present in 47% of the transplanted patients and

Submitted November 25, 2012; accepted January 31, 2013. Prepublished online as *Blood* First Edition paper, February 22, 2013; DOI 10.1182/blood-2012-11-469627.

Presented in part in abstract form at the 54th Annual Meeting of the American Society of Hematology, December 11, 2012, Atlanta, GA.

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

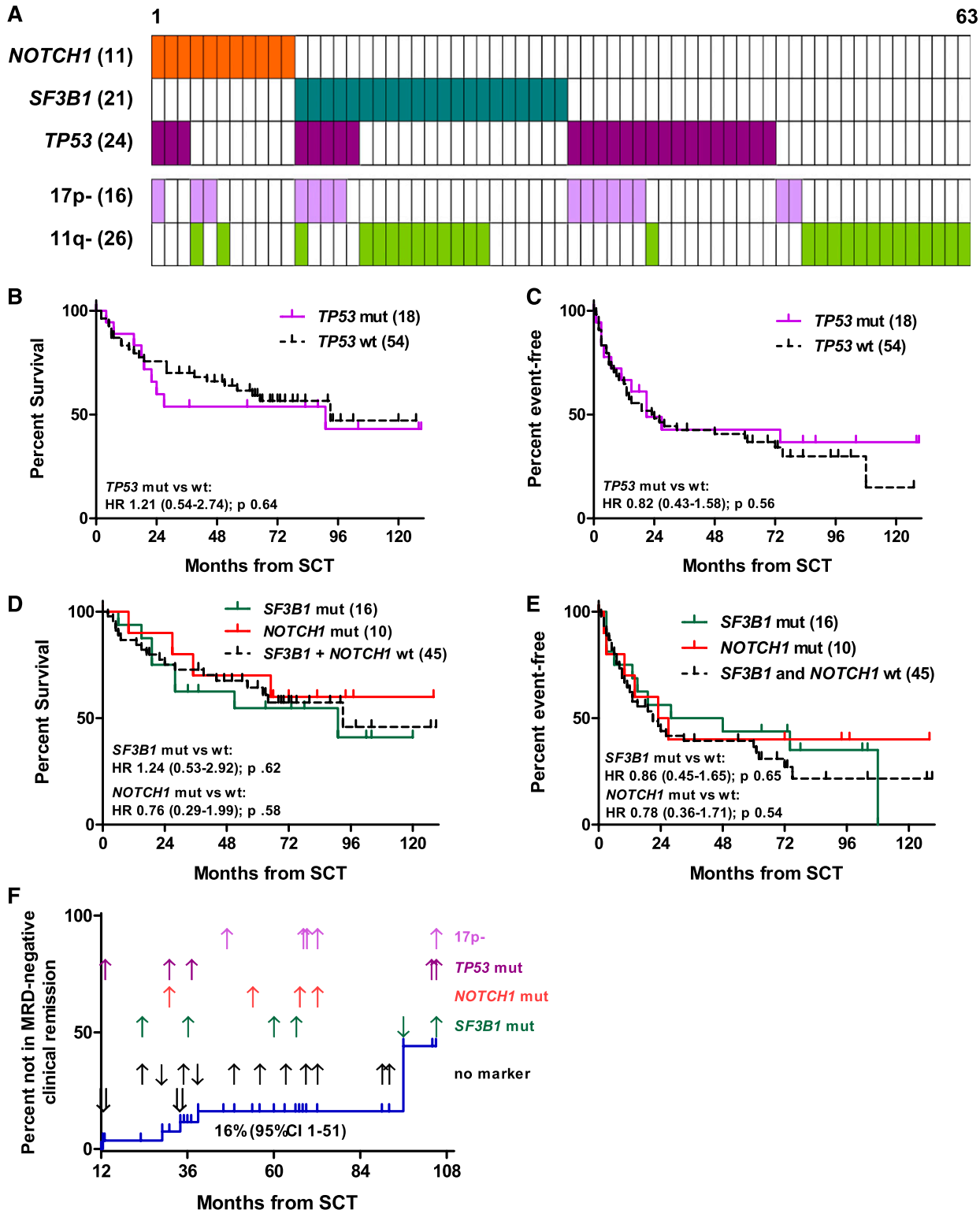

Figure 1. Genetic lesions and impact on clinical and MRD outcome. (A) Relationship of genetic lesions of all patients of the trial population (n = 100) who had mutational analysis available and at least a single mutation and/or 17p/11q deletion present (n = 63). Rows correspond to genes, and columns represent individual patients color-coded according to gene status (white, wild type; red, mutations of *NOTCH1*; dark green, mutations of *SF3B1*; dark purple, mutations of *TP53*; light purple, 17p deletion; light green, 11q deletion). (B, C) OS and EFS according to TP53 mutational status (purple, TP53-mutated with or without 17p- [n = 18]; black, TP53 wild-type with or without 17p- [n = 54]); (D, E) OS and EFS according to SF3B1 and NOTCH1 mutational status (green, SF3B1-mutated [n = 16]; red, NOTCH1-mutated [n = 10]; black, SF3B1 and NOTCH1 wild-type [n = 45]). (F) Durability of MRD-negative remission in 28 patients who had achieved this status at 12 months after HSCT. Both MRD reconversions and clinical relapses without prior MRD reconversion are counted as events. Patients are censored at the time of last available MRD measurement. Upward arrows indicate patients remaining MRD-negative and relapse-free at last measurement; downward arrows indicate patients with MRD reconversion. The patient reconverting to MRD positivity at +29 months is still in clinical CR 63 months post-HSCT; the other 2 relapsed subsequently. Downward double arrows indicate patients with no marker with clinical relapse from MRD negativity (one relapsed extranodally with rapidly fatal Richter's transformation; the other showed discrete lymph node enlargements that were considered as relapse without histological proof and responded to rituximab). Two patients are censored at 72 months (one 17p- and NOTCH1-mutated, the other without marker).

Table 1. Multivariate prognostic factor analyses for allografted patients

	Multivariate analysis			
Endpoint and variable	***P* value**	**HR**	**LowerCL**	**UpperCL**
Overall survival				
TCD vs no TCD	.004	3.46	1.49	8.04
Refractory at HSCT vs sensitive	.042	2.40	1.03	5.56
Fludara refractory vs sensitive	.13	2.61	0.75	9.16
Age (per year)	.36	0.98	0.93	1.03
TP53 mutated vs unmutated	.91	1.05	0.44	2.50
SF3B1 mutated vs unmutated	.60	0.79	0.33	1.88
NOTCH1 mutated vs unmutated	.53	0.69	0.22	2.16
Event-free survival				
TCD vs no TCD	.004	2.79	1.39	5.60
Refractory at HSCT vs sensitive	.002	3.13	1.53	6.38
Fludara refractory vs sensitive	.93	1.04	0.46	2.32
Age (per year)	.22	0.98	0.94	1.02
TP53 mutated vs unmutated	.25	0.66	0.32	1.34
SF3B1 mutated vs unmutated	.21	0.63	0.32	1.29
NOTCH1 mutated vs unmutated	.42	0.68	0.27	1.74

Missing values (TCD 0; Refractory/ sensitive at HSCT 1; Fludara refractory/ sensitive 31; age 0; *TP53*/17p abn 17; *SF3B1* mut 19; *NOTCH1* mut 19) were considered as individual category for each covariate. Results of missing value categories are not included in the table.

CL, confidence limit; TCD, in vivo T-cell depletion with alemtuzumab.

absent in 18%. The rest (35%) of the patients were not evaluable for fludarabine resistance because they underwent HSCT shortly after fludarabine exposure. Twenty-four percent of the patients had refractory CLL at HSCT. In vivo T-cell depletion (TCD) with alemtuzumab during conditioning was applied in 13% of the patients.

Genomic aberrations were assessed by fluorescence in-situ hybridization, as previously described.[18] *NOTCH1* was studied by direct sequencing of a polymerase chain reaction fragment from the PEST domain (exon 34, chr9:139 390,619-139 391,290).[12] *SF3B1* (exons 13 to 16) and *TP53* (exons 4 to 10) were analyzed by denaturing high-performance liquid chromatography (WAVE 3500HT, Transgenomic Inc) with subsequent sequencing of aberrant fragments, if needed, after subcloning.[9,11] Minimal residual disease (MRD) was monitored by MRD flow or allele-specific oligonucleotide primer *IGHV* real-time quantitative polymerase chain reaction.[19]

Fisher's exact test was used to compare categorical factors between groups of patients. Survival time data were calculated using the Kaplan-Meier method for overall survival (OS) and event-free survival (EFS), and cumulative incidence estimates were calculated in a competing risk framework for relapse and nonrelapse mortality (NRM). Kaplan-Meier curves were compared by logrank testing. Proportional hazards models (Cox regression) were fitted to investigate effects of prognostic factors for OS and EFS.[6] Data were analyzed as of June 30, 2012.

Results and discussion

With a median observation time of 6.0 years (range, 0.6 to 10.8 years), 6-year OS of all 100 patients enrolled was 53% (95% confidence interval [CI], 42% to 63%). The 90 allografted patients had a 6-year OS, EFS, relapse incidence, and NRM of 58% (95% CI, 48% to 69%), 38% (95% CI, 27% to 48%), 46% (95% CI, 34% to 58%), and 23% (95% CI, 13% to 32%), respectively (supplementary Figure 1). In comparison with the previous analysis, with 38 transplanted patients living event-free at a median follow-up time of 3.8 years,[6] no additional nonrelapse deaths but 6 additional relapse events (all from clinical CR; 2 of them in patients who tested MRD-negative at the 12-month landmark) occurred during the 2.2 years of additional observation time covered by the present study.

TP53, SF3B1, and *NOTCH1* mutational status each could be obtained in 80 of 100 patients, and fluorescence in-situ hybridization results were available in 82 of 100 patients. Mutations of *TP53*, *SF3B1*, and *NOTCH1* were found in 30%, 26%, and 14% of patients, respectively. These frequencies appear to be slightly higher than reported for patients with less-advanced disease.[9-14,16] Whilst *SF3B1* and *NOTCH1* mutations were mutually exclusive, 24% and 27% of patients with *SF3B1* and *NOTCH1* mutations also had a *TP53* mutation, respectively (Figure 1A). Genomic aberrations were evenly distributed except for overrepresentation of 17p- (46% positive) and underrepresentation of 11q- (4% positive) in the *TP53*-mutated subset and for overrepresentation of 11q- in the *SF3B1*-mutated subset (48% positive) (Figure 1A and supplementary Table 1). In contrast to observations from unselected CLL samples, trisomy 12 was not enriched in the *NOTCH1*-mutated subset in this high-risk subset of patients with CLL.[12,14]

By univariate comparison, OS of all 100 patients accrued was not significantly different between mutated and wild-type patients for *TP53* (hazard ratio [HR], 1.61; 95% CI, 0.8 to 3.23), *SF3B1* (HR, 1.43; 95% CI, 0.68 to 2.99), and *NOTCH1* (HR, 0.78; 95% CI, 0.34 to 1.84). When only the 90 allografted patients were considered, OS and EFS of mutated vs wild-type patients were not significantly different for *TP53*, *SF3B1*, and *NOTCH1* (Figure 1B-E). In *TP53*-mutated and *SF3B1*-mutated cases, the additional presence of 17p- and 11q-, respectively, had no significant effect on OS and EFS (supplementary Figure 2).

Multivariate analysis using Cox regression modeling including age, TCD, fludarabine resistance, and refractory disease at HSCT, in addition to the gene mutations as potential prognostic factors, did not show a significant effect of *TP53*, *SF3B1*, and *NOTCH1* mutations on OS and EFS (Table 1). Similarly, when *TP53*mut and/or 17p- was introduced as a covariate in the model, instead of *TP53*mut, to assess the overall effect of *TP53* abnormalities, a significant effect did not emerge (supplementary Table 2). In contrast, alemtuzumab TCD and refractory disease at HSCT retained their adverse effect on OS and EFS, as already observed in the previous analysis.[6]

Of 52 patients who were available for longitudinal MRD monitoring, 28 (54%) were relapse-free and MRD-negative 12 months after HSCT (including a single patient experiencing a clonally unrelated diffuse large-cell lymphoma 9 months after HSCT). The probability of being in MRD-negative remission 1 year after HSCT was not significantly different between patients with *TP53* (42%), *SF3B1* (54%), and *NOTCH1* (50%) mutations. During follow-up, 3 of the 28 MRD-negative patients showed MRD recurrence (in 2 of them subsequently followed by clinical relapse), and 2 other patients had clinical relapse without prior MRD reconversion. Only 1 of these 5 events occurred in an (*SF3B1*-) mutated patient. In contrast, 23 patients (82%) remained in MRD-negative clinical remission of CLL throughout the whole follow-up (Figure 1F).

In summary, late NRM was absent in the trial population, and only a few new relapses occurred during extended follow-up. These findings are in line with the 5-year follow-up data of the Seattle study,[4] as well as with some large, single-center studies with shorter observation times.[7,20] The risk for clinical CLL recurrence was particularly low in the subset of patients who had achieved MRD negativity at the 12-month post-HSCT landmark, and more than 80% of MRD-negative patients remained so throughout the whole follow-up, indicating a sustained effect of the graft vs leukemia activity conferred with HSCT in poor-risk CLL. This is in contrast to the MRD recurrence patterns observed with chemotherapy and antibody-based strategies in CLL.[21,22] However, the fact that 2

patients reconverted to MRD positivity beyond 3 years and subsequently relapsed suggests that MRD negativity at 12 months may not indicate definite CLL eradication in every case.

Moreover, for the first time we were able to show that after reduced-intensity HSCT, long-term clinical disease control and durable MRD negativity do not appear to be affected by either the presence of *TP53* mutation or the presence of *SF3B1* and *NOTCH1* mutations. Confirmation of these observations by prospective studies or post hoc analyses of completed trials is warranted.

Acknowledgment

This work was supported by grants from the Deutsche Jose-Carreras Leukämiestiftung e.V. Projects R02/18 and R05/02 and by grants from the Else Kröner Fresenius Stiftung (Else Kröner Forschungskolleg Ulm), the CLL Global Research Foundation (Alliance), the Virtual Helmholtz Institute (VH-VI-404, TP2), and the DFG (SFB 1074 project B2).

Authorship

Contribution: P.D. and S.S. designed and performed research, provided patients and samples, gave administrative support, analyzed data, and wrote the paper; T.Z. provided patients and samples, contributed analytical tools, and analyzed data; S.B., A.S., M. Rossi, P.P., A.B., and M. Ritgen contributed analytical tools and analyzed data; S.D. and R.B. analyzed data; U.H., D.B., M.Z., M.S., L.U., and C.S. provided patients and samples; M.H. provided patients and samples and gave administrative support; M.K. provided patients and samples, gave administrative support, contributed analytical tools, and analyzed data; N.S. designed research; H.D. designed research, gave administrative support, and helped write the paper. All authors proofread the paper and agreed to the data presented.

Conflict-of-interest disclosure: P.D. has received consulting fees from Roche, honoraria from Bayer, Roche, and Novartis, and research funding from Bayer; M.K. has received consulting fees from Roche and research funding from Roche and Novartis; S.S. has received consulting fees from Amgen, Bayer, and Roche, honoraria from Amgen, Bayer, Celgene, GlaxoSmithKline, and Roche, and research funding from Amgen, Bayer, Celgene, GlaxoSmithKline, and Roche; S.B. has received honoraria and research funding from Roche. The remaining authors declare no competing financial interests.

A complete list of the members of the GCLLSG CLL3X study group appears in "Appendix."

Correspondence: Peter Dreger, Department of Medicine V, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany; e-mail: peter.dreger@med.uni-heidelberg.de.

Appendix: study investigators

Additional members of the GCLLSG CLL3X trial study group are: Lutz Uharek (Charite Benjamin Franklin, Berlin, Germany); Dietrich Beelen (Universitätsklinikum, Essen, Germany); Bertram Glass (Universitätsklinikum, Göttingen, Germany); Norbert Schmitz and Matthias Zeis (Asklepios Klinik St. Georg, Hamburg, Germany); Bernd Hertenstein, Michael Stadler, and Matthias Eder (Medizinische Hochschule, Hannover, Germany); Peter Dreger and Ute Hegenbart (Innere Medizin V, Heidelberg, Germany); Jörg Schubert (Universitätsklinikum, Homburg, Germany); N. Basara (Idar-Oberstein, Germany, Klinik für KMT); Martin Gramatzki and Michael Kneba (II. Medizinische Klinik, Kiel, Germany); Michael Hallek and Christof Scheid (Universitätsklinikum, Köln, Germany); Andreas Burchert (Universitätsklinikum, Marburg, Germany); Sandra Cohen (Hopital Maisonneuve Rosemont, Montreal, Canada); Dietger Niederwieser (Universitätsklinikum, Leipzig, Germany); Ernst Holler (Universitätsklinikum, Regensburg, Germany); Donald Bunjes, Stephan Stilgenbauer, and Hartmut Döhner (III. Medizinische Klinik, Ulm, Germany).

References

1. Delgado J, Thomson K, Russell N, et al; British Society of Blood and Marrow Transplantation. Results of alemtuzumab-based reduced-intensity allogeneic transplantation for chronic lymphocytic leukemia: a British Society of Blood and Marrow Transplantation Study. *Blood.* 2006;107(4):1724-1730.
2. Tsimberidou AM, Keating MJ. Treatment of fludarabine-refractory chronic lymphocytic leukemia. *Cancer.* 2009;115(13):2824-2836.
3. Schetelig J, van Biezen A, Brand R, et al. Allogeneic hematopoietic cell transplantation for chronic lymphocytic leukemia with 17p deletion: a retrospective European Group for Blood and Marrow Transplantation analysis. *J Clin Oncol.* 2008;26(31):5094-5100.
4. Sorror ML, Storer BE, Sandmaier BM, et al. Five-year follow-up of patients with advanced chronic lymphocytic leukemia treated with allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. *J Clin Oncol.* 2008;26(30):4912-4920.
5. Farina L, Carniti C, Dodero A, et al. Qualitative and quantitative polymerase chain reaction monitoring of minimal residual disease in relapsed chronic lymphocytic leukemia: early assessment can predict long-term outcome after reduced intensity allogeneic transplantation. *Haematologica.* 2009;94(5):654-662.
6. Dreger P, Döhner H, Ritgen M, et al; German CLL Study Group. Allogeneic stem cell transplantation provides durable disease control in poor-risk chronic lymphocytic leukemia: long-term clinical and MRD results of the German CLL Study Group CLL3X trial. *Blood.* 2010;116(14):2438-2447.
7. Khouri IF, Bassett R, Poindexter N, et al. Nonmyeloablative allogeneic stem cell transplantation in relapsed/refractory chronic lymphocytic leukemia: long-term follow-up, prognostic factors, and effect of human leukocyte histocompatibility antigen subtype on outcome. *Cancer.* 2011;117(20):4679-4688.
8. Döhner H, Fischer K, Bentz M, et al. *p53* gene deletion predicts for poor survival and non-response to therapy with purine analogs in chronic B-cell leukemias. *Blood.* 1995;85(6):1580-1589.
9. Zenz T, Eichhorst B, Busch R, et al. *TP53* mutation and survival in chronic lymphocytic leukemia. *J Clin Oncol.* 2010;28(29):4473-4479.
10. Gonzalez D, Martinez P, Wade R, et al. Mutational status of the *TP53* gene as a predictor of response and survival in patients with chronic lymphocytic leukemia: results from the LRF CLL4 trial. *J Clin Oncol.* 2011;29(16):2223-2229.
11. Rossi D, Bruscaggin A, Spina V, et al. Mutations of the *SF3B1* splicing factor in chronic lymphocytic leukemia: association with progression and fludarabine-refractoriness. *Blood.* 2011;118(26):6904-6908.
12. Rossi D, Rasi S, Fabbri G, et al. Mutations of *NOTCH1* are an independent predictor of survival in chronic lymphocytic leukemia. *Blood.* 2012;119(2):521-529.
13. Quesada V, Conde L, Villamor N, et al. Exome sequencing identifies recurrent mutations of the splicing factor *SF3B1* gene in chronic lymphocytic leukemia. *Nat Genet.* 2012;44(1):47-52.
14. Balatti V, Bottoni A, Palamarchuk A, et al. *NOTCH1* mutations in CLL associated with trisomy 12. *Blood.* 2012;119(2):329-331.
15. López C, Delgado J, Costa D, et al. Different distribution of *NOTCH1* mutations in chronic lymphocytic leukemia with isolated trisomy 12 or associated with other chromosomal alterations. *Genes Chromosomes Cancer.* 2012;51(9):881-889.
16. Wang L, Lawrence MS, Wan Y, et al. *SF3B1* and other novel cancer genes in chronic lymphocytic leukemia. *N Engl J Med.* 2011;365(26):2497-2506.
17. Dreger P, Corradini P, Kimby E, et al; Chronic Leukemia Working Party of the EBMT. Indications for allogeneic stem cell transplantation in chronic lymphocytic

leukemia: the EBMT transplant consensus. *Leukemia.* 2007;21(1):12-17.

18. Döhner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med.* 2000; 343(26):1910-1916.

19. Böttcher S, Stilgenbauer S, Busch R, et al. Standardized MRD flow and ASO *IGH* RQ-PCR for MRD quantification in CLL patients after rituximab-containing immunochemotherapy: a comparative analysis. *Leukemia.* 2009;23(11):2007-2017.

20. Brown JR, Kim HT, Armand P, et al. Long-term follow-up of reduced-intensity allogeneic stem cell transplantation for chronic lymphocytic leukemia: prognostic model to predict outcome. *Leukemia.* 2013;27(2): 362-369.

21. Moreton P, Kennedy B, Lucas G, et al. Eradication of minimal residual disease in B-cell chronic lymphocytic leukemia after alemtuzumab therapy is associated with prolonged survival. *J Clin Oncol.* 2005;23(13): 2971-2979.

22. Bosch F, Ferrer A, Villamor N, et al. Fludarabine, cyclophosphamide, and mitoxantrone as initial therapy of chronic lymphocytic leukemia: high response rate and disease eradication. *Clin Cancer Res.* 2008; 14(1):155-161.