Graft-versus-leukemia activity may overcome therapeutic resistance of chronic lymphocytic leukemia with unmutated immunoglobulin variable heavy-chain gene status: implications of minimal residual disease measurement with quantitative PCR

Matthias Ritgen, Stephan Stilgenbauer, Nils von Neuhoff, Andreas Humpe, Monika Brüggemann, Christiane Pott, Thorsten Raff, Alexander Kröber, Donald Bunjes, Richard Schlenk, Norbert Schmitz, Hartmut Döhner, Michael Kneba, and Peter Dreger

The aim of this study was to investigate if graft-versus-leukemia (GVL) activity conferred by allogeneic stem cell transplantation (allo-SCT) is effective in chronic lymphocytic leukemia (CLL) with unmutated VH gene status. The kinetics of residual disease (MRD) were measured by quantitative allele-specific immunoglobulin heavy chain (IgH) polymerase chain reaction (PCR) in 9 patients after nonmyeloablative allo-SCT for unmutated CLL. Despite an only modest decrease in the early posttransplantation phase, MRD became undetectable in 7 of 9 patients (78%) from day +100 onwards subsequent to chronic graft-versus-host disease or donor lymphocyte infusions. With a median follow-up of 25 months (range, 14-37 months), these 7 patients remain in continuous clinical and molecular remission.

Introduction

Recently, the mutational status of the variable region of the immunoglobulin heavy-chain gene (VH) has been established as a crucial prognostic factor in chronic lymphocytic leukemia (CLL). Whereas patients with somatic mutations of the VH gene are characterized by stable disease and long survival, an unmutated germ line VH configuration is associated with a dismal prognosis.\(^1\)\(^{-}\)\(^4\) Allogeneic stem cell transplantation (allo-SCT) can result in long-term disease control in a proportion of patients with resistant CLL,\(^5\)\(^{-}\)\(^9\) and there is some evidence for graft-versus-leukemia (GVL) activity after allo-SCT.\(^10\)\(^{-}\)\(^12\) To date, however, it is unknown whether GVL can be effective in patients with unmutated VH status. Information on this issue is particularly important, as allo-SCT in CLL is increasingly performed using nonmyeloablative conditioning,\(^13\)\(^{-}16\) implying that the contribution of GVL for disease control becomes even more essential. The purpose of the present study was to investigate whether the therapeutic resistance of unmutated CLL can be overcome by allo-SCT following nonmyeloablative conditioning (NST), and to compare the relative contributions of conditioning regimen and GVL to tumor control.

Study design

Patients and treatment protocols

Included in this study were consecutive patients from an ongoing prospective trial on NST in CLL\(^17\) who had an unmutated VH status and diagnostic material available for longitudinal quantitative molecular monitoring. Minimum follow-up time required was 12 months. Eligibility criteria were as follows: refractoriness or early relapse (within 12 months) after fludarabine-containing treatment or after autologous SCT (auto-SCT); Richter transformation; or high-risk disease as defined by an unfavorable genetic profile (del11q23 and/or del 17p13 and/or unmutated VH status)\(^18\) in the presence of progressive disease. Allogeneic peripheral blood stem cells from granulocyte colony-stimulating factor (G-CSF)–mobilized HLA-identical donors were infused after conditioning by daily fludarabine (30 mg/m\(^2\)) and cyclophosphamide (500 mg/m\(^2\)) over 5 days, followed by cyclosporin A (CSA) and short-course methotrexate for graft-versus-host disease (GVHD) prophylaxis. In the case of unrelated donors, antilymphocyte globulin was added. CSA was tapered between day +60 and +100 after transplantation, in the absence of GVHD. Donor lymphocyte infusions (DLIs) were administered from day +120 onwards in the case of incomplete chimerism or residual disease after CSA withdrawal. Patients from a prospective protocol on the value of auto-SCT after myeloablative conditioning in CLL who fulfilled similar criteria served as controls.\(^19\) Eligibility and treatment details of auto-SCT have been described elsewhere.\(^6\)\(^,\)\(^5\)\(^,\)\(^20\) Treatment protocols were approved by the responsible institutional review boards.

Molecular studies

Immunoglobulin heavy chain (IgH) sequencing, mutational status assessment,\(^3\)\(^,\)\(^4\) interphase cytogenetics,\(^18\) and chimerism studies\(^21\) were done as described previously. IgH real-time polymerase chain reaction (RQ-PCR) for MRD quantification was performed with an ABI PRISM 7700 thermal cycler (Applied Biosystems, Weiterstadt, Germany).\(^22\) DNA from samples...
with known CLL cell content as assessed by standard flow cytometry (CD19/CD5/CD23/CD20 coexpression) were serially diluted in pooled polyclonal DNA to generate at least 5 standards. Specificity and sensitivity was defined as the last dilution with target DNA detectable or, in case of nonspecific amplification, the dilution with cycle threshold (C_T) values at least one cycle lower than the lowest C_T value found in polyclonal DNA. Each PCR showed a standard curve correlation coefficient of at least 0.95 with a slope of 3.0 to 3.9, minimum sensitivity was 10^−5, and MRD level calculation was based on comparative C_T analysis between follow-up samples and standards. Normalization by parallel amplification of the albumin gene allowed calculating of sensitivity levels for negative samples.

### Results and discussion

Altogether, 9 patients with unmutated high-risk CLL who had undergone NST and 26 control patients who had undergone auto-SCT for unmutated CLL met the inclusion criteria for this study. NST patients had pretreatment characteristics similar to the auto-SCT group in terms of sex, age, VH homology, unfavorable fluorescence in situ hybridization (FISH) karyotype, lymphocyte doubling time (LDT), and stage, but were more heavily pretreated and had a longer time from diagnosis to transplantation (Table 1).

Peripheral blood MRD kinetics in the NST patients were characterized basically by a biphasic pattern with an only modest decrease in the early posttransplantation phase (median MRD level: 1.9 × 10^−2 [range, 2.9 × 10^−3-1.9 × 10^0] before NST versus 1.1 × 10^−2 [range, 1.8 × 10^−4-2.3 × 10^−2] at day +100; P = .002), followed by a marked reduction of more than 2 logs in the MRD levels between day +100 and day +200 (8.4 × 10^−5 [range, 1.0 × 10^−5-1.1 × 10^−2]; P = .005). During follow-up, MRD levels stayed low and became durably undetectable in 7 of 9 patients (78%; Figure 1). In 5 of the 9 NST patients, disappearance of RQ-PCR–measurable MRD occurred subsequent to development of extensive chronic GVHD. Three patients remained free of GVHD. Two of these patients converted to sustained MRD negativity after DLI without any GVHD, one without conversion to complete chimerism (Figure 1), whereas one patient failed to

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**Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th></th>
<th>NST group</th>
<th>Auto-SCT group</th>
<th>P</th>
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<tr>
<td>No.</td>
<td>9</td>
<td>26</td>
<td>NA</td>
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<tr>
<td>No. male/no. female</td>
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<td>21/5</td>
<td>NS*</td>
</tr>
<tr>
<td>Age, y (range)</td>
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<td>51 (34-60)</td>
<td>NS†</td>
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<tr>
<td>VH homology, % (range)</td>
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<td>100 (98.3-100)</td>
<td>NS†</td>
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<tr>
<td>FISH del 11q23 or del 17p13 (%)</td>
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<td>8/23 (35)</td>
<td>NS*</td>
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<tr>
<td>Lymphocyte doubling time less than 12 mos. (%)</td>
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<td>14/17 (82)</td>
<td>NS*</td>
</tr>
<tr>
<td>Binet stage B/C, no. (%)</td>
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<td>17 (88)</td>
<td>NS*</td>
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<tr>
<td>Time to ASCT, mos. (range)</td>
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<td>12 (3-117)</td>
<td>.01†</td>
</tr>
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<td>No. of previous regimens (range)</td>
<td>4 (1-6)</td>
<td>2 (1-4)</td>
<td>.009†</td>
</tr>
</tbody>
</table>

NS indicates not significant (significance level = .05); NA, not applicable.

* Determined using 2-tailed Fisher exact test.
† Determined using 2-tailed Mann-Whitney test.

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**Figure 1. MRD kinetics after autologous SCT and nonmyeloablative allogeneic SCT.** MRD levels (number of CLL-specific DNA copies per total DNA copies in the sample compared with the reference [pretherapeutic] sample) of patients after myeloablative conditioning and auto-SCT (A) and after nonmyeloablative allo-SCT (B). (●) denotes MRD-positive samples; (○) denotes the sensitivity of PCR-negative samples (calculated minimum MRD level, which would have been detected in this particular sample); and (△) denotes percentage of donor chimerism. (C–F) Individual MRD kinetics in correlation to cyclosporine withdrawal (C), DLI (D), and chronic GVHD (E). (F) Case with discordant MRD and chimerism data, implying that GVL can induce CLL clearance without achieving full donor chimerism.
eliminate MRD despite DLI. The remaining patient, who was the only carrier of a 17p13 deletion in this series, showed evidence of early clinical relapse with progressive bulbar lymphopenia prior to the onset of extensive chronic GVHD 4 months after allo-NST. Although GVHD was associated with declining but positive MRD levels, the patient died in progressive disease at month +19. All other 8 patients are in ongoing complete clinical remission with a median follow-up of 29 months (range, 14-41 months) after transplantation.

Auto-SCT resulted in an immediate reduction of MRD levels (6.4 × 10^−3 [range, 4.0 × 10^−3-3.9 × 10^−1] before SCT vs. 2.3 × 10^−4 [range, 5.5 × 10^−6-2.5 × 10^−3] at day +100; P = .0002) without further decrease during longer follow-up (median clone-specific DNA copy number at 2 years after transplantation was 5.0 × 10^−3 [range, 1.0 × 10^−3-6 × 10^−1]; P = .43 if compared to day +100). Although 6 of 26 patients (23%) eventually became PCR negative, there were only 2 patients whose MRD levels remained below the threshold of detection until their most recent follow-up at 28 and 29 months after transplantation, respectively (Figure 1). Clinical relapse, progression, or death was observed in 11 patients after auto-SCT, translating into a median progression-free survival of 48 months. Occurrence of clinical relapse was not predictable by day +100 MRD levels, day +360 MRD levels, or by achievement of MRD negativity at any time after transplantation.

There is circumstantial evidence that GVL mechanisms contribute to disease control after allo-SCT in CLL.6-8,12,16,17 However, using sensitive long-term MRD quantification, the present study is the first to demonstrate a clear correlation between CLL load kinetics and immune modulating maneuvers after NST. Our results show that profound and sustained complete molecular responses occur only after establishing chronic GVHD or DLI, whereas the influence of the conditioning regimen on the tumor cell load is limited.

Of note, all patients studied had an unmutated V_{H} gene status, showing for the first time that unmutated CLL is sensitive to GVL. Since the GVL-mediated antileukemic activity observed here is clearly superior to that of the intensified fludarabine-cyclophosphamide regimen used for conditioning as well as to the fully myeloablative treatment administered for auto-SCT, allo-SCT appears to be the most effective and yet only potentially curative modality available for treatment of unmutated CLL. This conclusion is confirmed by a recent preliminary report of clinical observations.21 In contrast, auto-SCT can reduce but not eradicate PCR-measurable tumor load in the majority of patients with unmutated CLL. This observation is in line with previous studies showing that complete responses documented by clinical criteria and by less-sensitive CDR3 consensus primer PCR assays are not durable after auto-SCT.4,12,20,23

In summary, this study demonstrates that allo-SCT can provide sustained disease control in unmutated CLL. Complete elimination of measurable MRD after allo-SCT is largely dependent on GVL, whereas the contribution of the conditioning regimen to treatment success appears to be limited. These results may help to define the future role of allo-SCT within the growing therapeutic arsenal for high-risk CLL.

Acknowledgments

The authors are grateful to the German CLL Study Group (DCLLSG; chairman Michael Hallek) and all the contributing centers taking care of patients being enrolled in this study. As in all studies of the DCLLSG, the cytogenetic studies were performed by S. Stilgenbauer, A. Krober, and H. Döhner at the University of Ulm, the MRD and chimerism analyses were performed by M. Ritgen, A. Humpe, and M. Kneba at the University of Schleswig-Holstein in Kiel.

References

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