A comparison of donor lymphocyte infusions or imatinib mesylate for patients with chronic myelogenous leukemia who have relapsed after allogeneic stem cell transplantation

Imatinib mesylate is highly effective in relapsed chronic myelogenous leukemia (CML) after allogeneic hematopoetic stem cell transplantation (HSCT). However, it is unknown whether imatinib produces durable molecular remissions. The outcome of CML patients transplanted at our center who had received only imatinib for relapse after HSCT was compared with that of patients treated with donor lymphocyte infusions (DLI). Imatinib therapy resulted in a higher incidence of relapse and inferior leukemia-free survival \( (p=0.006 \text{ and } p=0.016, \text{ respectively}) \). These data suggest that imatinib alone probably does not cure relapse after HSCT.

Key words: donor lymphocyte infusion, imatinib, chronic myelogenous leukemia, allogeneic stem cell transplantation, relapse.

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From the hematopoetic cell transplantation, Department of Medicine III, University of Munich, Klinikum Grosshadern, Ludwig Maximilians University of Munich and GSF-National Research Center for Environment and Health, Munich, Germany (MW, JT, GL, HJK); Munich Leukemia Laboratory, Munich, Germany (SS, CS).

Correspondence:
Martin Weisser, Department of Internal Medicine III, University of Munich, Klinikum Grosshadern, Marchioninistr. 15, 81377 Munich, Germany.
E-mail: martin.weisser@med.uni-muenchen.de

At present, donor lymphocyte infusion (DLI) is the standard therapy for patients with chronic myelogenous leukemia (CML) relapsing after allogeneic hematopoetic stem cell transplantation (HSCT). DLI has proven to restore full donor chimerism and produce long-term complete molecular genetic remissions. However, possible side effects, such as graft-versus-host disease (GvHD) and myelosuppression lead to a considerable amount of treatment-related morbidity and mortality. Imatinib mesylate has shown remarkable short-term efficacy in the treatment of CML combined with low toxicity. However, the follow-up of patients treated with imatinib is still rather short and it remains unclear whether this drug has the potential to create durable molecular remissions, maintain these responses after discontinuation of the drug, and thus lead to definite cure of relapse. We, therefore, retrospectively analyzed the long-term outcome of CML patients who had received imatinib alone for therapy of relapse after allogeneic HSCT at our center. The results were compared to the outcome of patients who were treated with DLI as relapse therapy.

Design and Methods

Patients

We evaluated patients with BCR-ABL positive CML who had undergone allogeneic HSCT in chronic or accelerated phase and who had relapsed after the transplant. Patients had received either DLI or imatinib for treatment of relapse. Imatinib was given in cases in which DLI was difficult to obtain, explaining the larger proportion of unrelated donors in the imatinib group. Thirty-one CML patients were evaluable, of whom 21 were treated with DLI and ten with imatinib. The median follow-up time was 1230 days (range 180-3530 days). Imatinib was administered at a dose of 400 mg-800 mg/day depending on response. Imatinib was escalated in 200 mg steps if there was an unsatisfactory response. An unsatisfactory response was defined as stable polymerase chain reaction (PCR) ratios in three consecutive independent PCR samples within one log of the baseline level in two consecutive samples, or signs of hematologic progress. The median duration of imatinib therapy was 365 days (range 45-1530). The median number of DLI was 1.5 per patient (range 1-3). The CD3 cell concentration ranged from \( 5 \times 10^{7} \text{ - } 1 \times 10^{8} / \text{kg} \). The initial cell dose was aimed to be \( 1 \times 10^{7} / \text{kg} \) for unrelated donors and \( 1 \times 10^{7} / \text{kg} \) for sibling donors. Lower initial cell doses were administered to patients with a history of severe acute GvHD after allogeneic HSCT. Patients received up to three infusions of donor lymphocytes, with escalating cell doses, at intervals of 4-6 weeks provided there were no clinical signs of GvHD. The patients’ characteristics are shown in Table 1.

Molecular cytogenetic and molecular genetic investigations

In order to evaluate response to therapy, routine fluorescent in situ hybridization (FISH) analysis for BCR-ABL-rearrangement...
was performed in all cases on interphase nuclei from bone marrow smears. The background level for FISH analysis was 5%. For further details of the techniques used, refer to the descriptions by Schoch et al.³

### Nucleic acid isolation

Mononuclear cells were obtained by Ficoll density gradient centrifugation. For each sample mRNA from 5×10⁶ human cells was isolated according to the Magna Pure LC mRNA protocol for human cells (Roche Diagnostics, Mannheim, Germany) and eluted in 30μL of H₂O.

### cDNA synthesis

Random primed cDNA synthesis was performed with Superscript II (Invitrogen, San Diego, Ca, USA) on diagnostic samples with 5μL of mRNA corresponding to approximately 0.8×10⁶ cells in a 50μL reaction.

### Quantitative real-time PCR

Quantitative PCR was performed using the LightCycler® System (Roche Diagnostics, Mannheim, Germany) applying hybridization probes as the detection format. The PCR conditions, primers and hybridization probes used to amplify and quantify the BCR-ABL fusion transcripts were as described by Emig et al.⁶ The expression of BCR-ABL was normalized against the expression of the control gene ABL to adjust for variations in RNA quality and efficiencies of cDNA synthesis. The expression ratios are given as: 100×BCR-ABL/ABL expression was quantified according to Emig et al.⁶ In addition, qualitative nested RT-PCR was performed as reported by Maurer et al.⁷

### Definition of relapse to imatinib or DLI therapy

Relapse was defined as: (i) primary hematologic/cytogenetic/molecular unresponsiveness or (ii) detection of increasing percentages of Philadelphia-chromosome positive metaphases and/or BCR-ABL positive inter-

### Table 1. Patients’ characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Imatinib (n=10)</th>
<th>DLI (n=21)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>33 (26-57)</td>
<td>37 (22-59)</td>
<td>0.452</td>
</tr>
<tr>
<td>Sex</td>
<td>6 m/4 f</td>
<td>13 m/8 f</td>
<td>1.00</td>
</tr>
<tr>
<td>Stage at HSCT</td>
<td>Chronic phase</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>Accelerated phase</td>
<td>3</td>
<td>4</td>
<td>0.252</td>
</tr>
<tr>
<td>Donor</td>
<td>Sibling</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Unrelated</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>HMD-prophylaxis</td>
<td>CsA/MTX</td>
<td>n=10</td>
<td>n=21</td>
</tr>
<tr>
<td>Median time to 1st relapse (days)</td>
<td>849 (104-6205)</td>
<td>692 (364-2323)</td>
<td>0.704</td>
</tr>
<tr>
<td>Type of relapse</td>
<td>Cytogenetic</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Hematologic</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Median time to 2nd relapse (days)</td>
<td>431 (45-1460)</td>
<td>730 (122-991)</td>
<td>0.656</td>
</tr>
</tbody>
</table>

CsA: cyclosporine A; MTX: methotrexate.

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values were less than 0.05. Using Kaplan-Meier estimates, curves were calculated for the probability of second relapse, overall survival, and leukemia-free survival from the start of therapy for the first relapse after allogeneic HSCT. The curves were compared using a double sided log rank test. Results were statistically significant at a level of p<0.05 at both sides.

### Results and Discussion

#### Best response to therapy

After DLI therapy 20/21 patients (95%) achieved a complete molecular remission. Imatinib therapy resulted in hematologic, complete molecular cytogenetic, and complete molecular genetic remission in 9/10 (90%), 7/10 (70%), and 7/10 (70%) patients, respectively. There was a trend towards a higher rate of molecular remissions assessed by real time RT-PCR for the DLI group (p=0.087). The time to achieve molecular complete remission was 7.5 months in the DLI group and 6 months in the imatinib group (p=1.0). BCR-ABL was undetectable by qualitative nested RT-PCR in 81% (17/21) of the DLI group and 50% (5/10) of the imatinib group (p=0.115).

#### Relapse analysis

Among the ten patients treated with imatinib, relapse occurred in six (60%) patients while they were receiving imatinib therapy. In four patients imatinib was discontinued after achievement of complete molecular remissions. These four patients had received imatinib for at least 1 year (n=2), 2 years (n=1) or 3 years (n=1). Complete molecular remission had been ascertained in at least two consecutive samples from all of these patients before imatinib was discontinued. Only one of these four patients has remained in molecular remission, now lasting 2 years, after withdrawal of imatinib. The other three patients relapsed 2 (n=1) and 4 (n=2) months after cessation of imatinib. For therapy of second relapse imatinib was restarted in two patients, resulting in a complete molecular cytogenetic remission. The other seven patients received DLI, resulting in a complete molecular cytogenetic remission in six of them. One patient was refractory to DLI and underwent a second HSCT after which a complete molecular remission was achieved. Three patients (14%) relapsed after DLI. Two of these patients relapsed after achievement of a molecular remission. The other patient showed no molecular cytogenetic or molecular genetic response to DLI. The probability of relapse was signif-

### Statistics

The SPSS 12.0 for Windows software package (SPSS, Chicago, IL, USA) was used for statistical analysis. Fisher’s exact test was performed when applicable to analyze differences between cohorts of patients. Differences were statistically significant when two-sided p values were less than 0.05. Using Kaplan-Meier estimates, curves were calculated for the probability of second relapse, overall survival, and leukemia-free survival from the start of therapy for the first relapse after allogeneic HSCT. The curves were compared using a double sided log rank test. Results were statistically significant at a level of p<0.05 at both sides.
significantly higher in the imatinib group than in the DLI group ($p=0.006$; Figure 1).

**GvHD**

GvHD grades II-IV requiring immunosuppressive therapy occurred in 11/21 (52%) patients after DLI therapy. Seven patients developed extensive chronic GvHD. Resolution of GvHD was observed in four patients. No GvHD reaction was seen after imatinib therapy.

**Leukemia–free survival and overall survival**

The rate of leukemia-free survival was significantly higher in the DLI group than in the imatinib group ($p=0.016$; Figure 2). Three patients died in the DLI group, two of GvHD and one of progressive leukemia. The rate of treatment-related mortality was, therefore, 10% in the DLI group whereas there were no treatment-related deaths in the imatinib group ($p=0.533$). The probability of overall survival at 5 years was 100% in the imatinib group and 76% in the DLI group ($p=0.183$). We retrospectively compared the long-term outcome of patients treated with imatinib or DLI for relapse after allogeneic HSCT. There was a trend towards higher rates of complete molecular remissions in the DLI-treated group. Among the ten patients treated with imatinib six (60%) relapsed under ongoing imatinib therapy. Another three patients relapsed after discontinuation of imatinib. Only one patient remained in molecular remission after imatinib withdrawal. This observation suggests that despite molecular responses, documented over 2 years at longest, it is unlikely that patients can be cured of CML by imatinib alone and that cellular therapy for relapse after HSCT is inevitable. This observation is in line with a report by Cortes and co-workers demonstrating rapid relapse in conventionally treated CML patients after discontinuation of imatinib. The higher relapse rate in the imatinib group also resulted in an inferior leukemia-free survival in these patients as compared to those treated with DLI. Studies on the treatment of relapse with DLI have previously shown that DLI from unrelated donors are associated with lower response rates and a higher risk of treatment-related mortality. Thus, although the differences in donor distribution of the present study were not statistically significant they may have influenced the outcome in favor of the DLI group. Acute GvHD was observed in more than half of the patients treated with DLI and led to extensive chronic GvHD in one third of the patients. In total, three patients died after DLI – two of GvHD, and one of progressive disease. The rate of treatment-related mortality was 10% in the DLI group and 0% in the imatinib group. Thus there was a trend towards a better overall survival in the imatinib group. However, overall survival figures should be interpreted cautiously as the follow-up was shorter in the imatinib group and, considering the high incidence of relapse, patients will subsequently receive DLI as a second treatment. Taking into account the limitations of the low numbers of patients studied, our data suggest that imatinib, in contrast to DLI, does not provide definite cure for relapsed CML after allogeneic HSCT. These observations need to be confirmed in more patients in prospective trials.

MW: data analysis, wrote the manuscript; JT: data analysis; SS: molecular genetic analysis; CS: cytogenetic analysis; GL: data analysis; HJK: Head of BMT-Unit, responsible author. The authors declare that they have no potential conflicts of interest. Manuscript received October 25, 2005. Accepted March 6, 2006.
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