In conclusion, this report presents the first use of RT-QPCR to accurately quantify the level of the DEK-CAN transcript in t(6;9)AML patients in sequential BM and PB samples from diagnosis to relapse. The protocol produces linear quantification of the DEK-CAN transcript over a wide range of levels. The level of the DEK-CAN transcript was more than $1 \times 10^{-2} \ DEK-CAN/ABL$ in all patients after induction chemotherapy. After two further courses of chemotherapy, the levels fell to less than $1 \times 10^{-4}$, which indicates that chemotherapy can reduce the leukemic load, but it remains persistently detectable, albeit at a low level. All three patients had a relatively high level of MRD prior to relapse, even though the samples were morphologically and cytogenetically normal. These data support the observations made by Soekerman et al. and Lillington et al. that t(6;9) patients respond poorly to chemotherapy alone with a median survival of less than one year.

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References


Acute Lymphoblastic Leukemia

The achievement of molecular complete remission during treatment with imatinib mesylate correlates with relapse-free survival in bcr/abl-positive acute lymphoid leukemia patients

Using quantitative reverse-transcription polymerase chain reaction we investigated the significance of achieving molecular complete remission (CR) in 12 patients with bcr/abl-positive acute lymphocytic leukemia treated with imatinib. The 6 patients who achieved molecular CR had significantly better relapse-free survival (RFS) than the others (9 vs 4 months) ($p=0.000$). Moreover, the 6 patients with a bcr-abl/GAPDH×100,000 ratio <2 after 4 weeks of treatment had significantly better RFS (10.5 vs 4 months) ($p=0.004$).

Recently, the abl protein tyrosine-kinase inhibitor imatinib mesylate has been shown to induce complete responses in 60% to 70% of patients with relapsed/refractory Philadelphia-positive acute lymphoid leukemia (ALL). Unfortunately, the median time to progression of all patients is only 2 to 2.5 months and that of patients who respond still only 3 months because of the development of resistance to imatinib. Only a minority of patients remain in remission for longer periods. Interestingly, it has been shown that the amount of minimal residual disease (MRD) after 4 weeks of treatment correlates with relapse-free survival (RFS) in these patients.

Moreover, the velocity and magnitude of the increase of bcr/abl levels correlates with RFS and overall survival (OS). Nevertheless, only few data are available concerning the ability of imatinib to induce molecular complete remission (mCR) in Philadelphia-positive ALL patients, and the significance of achieving a mCR is not completely clear. We studied 12 bcr/abl-positive ALL patients, treated with imatinib at the daily dose of 400 mg (3 cases), 600 mg (7 cases), and 800 mg (2 cases), strictly monitoring the MRD by quantitative TaqMan reverse transcription polymerase chain reaction (RT-PCR). Two patients were treated while in first CR, 8 for relapsed/refractory disease and 2 were previously untreated. Ten out of the 12 patients were in CR after 4 weeks of imatinib therapy. Six achieved a mCR after 4-12 weeks; 5/10 then relapsed, 6 died of leukemia, 1 died while in CR, 3 are alive in CR, 1 is alive with disease (Table 1).

First, we investigated whether the achievement of a mCR correlated with RFS and OS. Six patients achieved a mCR, six did not. The median RFS and OS were 8 and 10.5 months in the former 6 patients and 4 and 5.5 months in the latter group, with a significant difference in terms of RFS ($p=0.0006$), but not of OS ($p=0.29$) (Figure 1). Notably, all the patients who achieved a mCR had a RFS of at least 8 months, whereas the others had a RFS not longer than 5 months. Taken together, these data show that the achievement of a mCR correlates with RFS, and the evaluation of MRD by quantitative RT-PCR allows discrimination between patients with a relatively better or worse prognosis.

Secondly, we investigated whether the MDR levels after 4 and 8 weeks of imatinib therapy correlated with RFS and OS. In particular, we considered patients with a bcr-abl/GAPDH×100,000 ratio 2 or >2. After 4 weeks of therapy, 4 patients had a small amount of MRD with a ratio <2 whereas 8 had a ratio >2. The median RFS and OS were, respectively, 10.5 and 11.5 months in the former group, and 4 and 9 months in the latter group. The difference was significant for RFS ($p=0.0067$). Analogous results were obtained considering the amount of MRD after 8 weeks of therapy. All the 3 patients with a bcr-abl/GAPDH×100,000 ratio <2 after 4 weeks of treatment achieved a mCR. Nevertheless, 2 patients with higher ratios at this time also later obtained a mCR. Taken together, these data confirm that the amount of MRD after 4 and 8 weeks of therapy is significant correlated with RFS.

Analogous results were obtained even if the patient who started the imatinib therapy while in molecular CR was
Letters to the Editor

Table 1. Treatment outcome and minimal residual disease evaluation.

<table>
<thead>
<tr>
<th>Disease status before treatment</th>
<th>Initial Imatinib dose</th>
<th>Response after 4 weeks</th>
<th>Best response</th>
<th>MRD after 4 weeks Bcr-abl/ GAPDH×10^6</th>
<th>MRD after 8 weeks Bcr-abl/ GAPDH×10^6</th>
<th>CR duration</th>
<th>Site of relapse</th>
<th>Survival duration</th>
<th>Status at last follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Molecular CR</td>
<td>800</td>
<td>Molecular-CR</td>
<td>Molecular-CR</td>
<td>0</td>
<td>14+</td>
<td>–</td>
<td>15+</td>
<td>A &amp; W</td>
<td></td>
</tr>
<tr>
<td>2 Cytogenetic CR</td>
<td>800</td>
<td>Cytogenetic-RC</td>
<td>Molecular-CR</td>
<td>1.8</td>
<td>0.23</td>
<td>8</td>
<td>Marrow (mol)</td>
<td>9</td>
<td>Dead/Leuk</td>
</tr>
<tr>
<td>3 Relapse</td>
<td>600</td>
<td>CR</td>
<td>CR</td>
<td>200</td>
<td>350</td>
<td>4</td>
<td>Marrow</td>
<td>5</td>
<td>Dead/Leuk</td>
</tr>
<tr>
<td>4 Relapse</td>
<td>600</td>
<td>Cytogenetic-RC</td>
<td>Molecular-CR</td>
<td>0</td>
<td>0</td>
<td>10+</td>
<td>Marrow</td>
<td>11+</td>
<td>A &amp; W</td>
</tr>
<tr>
<td>5 Relapse</td>
<td>600</td>
<td>Molecular-CR</td>
<td>Cytogenetic-CR</td>
<td>8.5</td>
<td>2.27</td>
<td>7/39+</td>
<td>Marrow</td>
<td>40+</td>
<td>A &amp; W</td>
</tr>
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<td>6 Relapse</td>
<td>600</td>
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<td>Cytogenetic-CR</td>
<td>12</td>
<td>1.1</td>
<td>8</td>
<td>Marrow</td>
<td>15</td>
<td>Dead/Leuk</td>
</tr>
<tr>
<td>7 Relapse</td>
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<td>Molecular-CR</td>
<td>3</td>
<td>1.22</td>
<td>8</td>
<td>Marrow</td>
<td>11</td>
<td>Dead/Leuk</td>
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<tr>
<td>8 Relapse</td>
<td>600</td>
<td>Molecular-CR</td>
<td>Molecular-CR</td>
<td>1</td>
<td>2</td>
<td>11+</td>
<td>Marrow (mol)</td>
<td>12+</td>
<td>A &amp; W</td>
</tr>
<tr>
<td>9 Relapse</td>
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<td>NR</td>
<td>–</td>
<td>100</td>
<td>100</td>
<td>–</td>
<td>10</td>
<td>Dead/Leuk</td>
<td></td>
</tr>
<tr>
<td>10 Relapse</td>
<td>400</td>
<td>NR</td>
<td>–</td>
<td>100</td>
<td>100</td>
<td>–</td>
<td>5</td>
<td>Dead/Leuk</td>
<td></td>
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<tr>
<td>11 Diagnosis</td>
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<td>280</td>
<td>245</td>
<td>4</td>
<td>Marrow</td>
<td>5</td>
<td>Dead/Leuk</td>
</tr>
<tr>
<td>12 Diagnosis</td>
<td>400</td>
<td>CR</td>
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<td>200</td>
<td>200</td>
<td>5</td>
<td>Marrow</td>
<td>8+</td>
<td>A &amp; Leuk</td>
</tr>
</tbody>
</table>

NR: resistant; *p*; persisting condition; marrow (mol): molecular bone marrow relapse; †this patient obtained a molecular CR after the addition of α interferon; A & W: alive and well; dead/leuk-MOF: dead for leukemia progression-multi organ failure.

Figure 1. The achievement of molecular complete remission significantly correlates with RFS (A) but not with OS (B).

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Table 1. Treatment outcome and minimal residual disease evaluation.

In our series, RFS and OS were remarkably longer than in that report. In particular, the median RFS was 8 months (vs 2.2), and the median OS was 10.5 months (vs 4.9). These differences can be explained both by the limited number of patients in our study, and by the different treatment schedules, in our cases including intrathecal prophylaxis. This element could be relevant, since a significant rate of central nervous system relapses was reported in other series and imatinib has been shown to be unable to cross the brain-blood barrier. Finally, as in our study, Scheuring and colleagues did not find significant correlations between MRD and OS; maybe this was due to the puzzling effect of the addition of further therapies at relapse.

We conclude that MRD monitoring by quantitative RT-PCR during imatinib treatment may allow bcr/abl positive ALL patients with relatively different prognoses to be identified and may improve the patients' management. However, imatinib does not appear to be curative in ALL, and further treatments should be promptly planned whenever possible.

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Key words: molecular complete remission, acute lymphocytic leukemia, imatinib.

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excluded. Recently, Scheuring and colleagues demonstrated that after 4 and 8 weeks of imatinib treatment correlates with RFS. Moreover, they demonstrated the usefulness of serial MRD analyses for predicting response duration. Nevertheless, only a minority of patients obtained a mCR, and the specific significance of achieving a mCR could not be estimated. In our series, RFS and OS were remarkably longer than in that report. In particular, the median RFS was 8 months (vs 2.2), and the median OS was 10.5 months (vs 4.9). These differences can be explained both by the limited number of patients in our study, and by the different treatment schedules, in our cases including intrathecal prophylaxis. This element could be relevant, since a significant rate of central nervous system relapses was reported in other series and imatinib has been shown to be unable to cross the brain-blood barrier. Finally, as in our study, Scheuring and colleagues did not find significant correlations between MRD and OS; maybe this was due to the puzzling effect of the addition of further therapies at relapse.

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Letters to the Editor

Acute Lymphoblastic Leukemia

Molecular detection of minimal residual disease is associated with early relapse in adult acute lymphoblastic leukemia

Several studies in childhood acute lymphoblastic leukemia (ALL) have documented that molecular detection of minimal residual disease (MRD) based on screening for T-cell receptor and immunoglobulin gene rearrangements can identify patients at a high risk of relapse. In our experience, evaluation of MRD in adult ALL can help to identify high risk patients.

Several studies in childhood acute lymphoblastic leukemia (ALL) have demonstrated the strong association between minimal residual disease (MRD) level and the risk of relapse. The most useful methods for MRD monitoring currently available are polymerase chain reaction (PCR) techniques, using either chromosome aberrations that result in fusion gene transcripts or patient-specific junctional regions of rearranged immunoglobulin (Ig) and T-cell receptor (TCR) genes, and flow cytometric detection of aberrant immunophenotypes. The use of antigen-receptor gene rearrangements has allowed detection of the presence of MRD, overcoming the diagnostic problem posed by the lack of recurrent chromosomal abnormalities (such as t(9;22) and t(4;11)) in most patients with ALL.

The value of MRD detected by PCR and/or immunologic techniques has been extensively evaluated in childhood ALL but less studied in adult ALL. The aim of the present study was to determine the value of molecular MRD monitoring in predicting outcome in adult ALL patients.

We evaluated 29 patients (10 males and 19 females; median age 27 years (range 15 to 61); 23 with common ALL, 2 with pro-B ALL, and 4 with T-ALL) admitted to our Institution from April 1996 to March 2001. They were treated with the multicenter co-operative GIMEMA (Gruppo Italiano Malattie Ematologiche dell’Adulto) LAL 0496 protocol; all patients achieved hematologic complete remission (CR) after induction therapy.

The bone marrow samples collected at diagnosis included more than 90% of blasts. The MRD analysis was performed at the following time points: post-induction, post-consolidation, every three months for the first year and every six months thereafter. We investigated the MRD level using rearrangements of the genes for T-cell receptors γ (TCRγ), δ (TCRδ) and Igκ (Igκ) as molecular markers for the leukemic clone and heteroduplex PCR analysis to detect the molecular marker. Sensitive detection required determination of the sequence of the marker gene from material obtained at diagnosis, and development of a specific probe that recognizes the leukemic clone, with a minimal target sensitivity of 10−7/10−8. The concentration of leukemic cells in the bone marrow samples during follow-up was estimated by comparison of the signals with those of 10-fold dilution samples (10−1 to 10−7) of the DNA at diagnosis. This resulted in a reproducible semiquantitative estimation of MRD. A cut-off value of 1/106 was used to divide patients into two categories: MRD positive (> 10−7) and MRD negative (< 10−8). The former value indicated molecular relapse whereas the latter defined patients in molecular remission. In all cases evaluated for MRD at least two clonal rearrangements were found.

At the post-induction and post-consolidation points, and after three months of maintenance, 14 patients (48%) were MRD negative. Two of these cases, despite having undetectable disease during the molecular follow up, developed isolated central nervous system (CNS) relapse after 7 and 9 months; in both cases the extramedullary localization of ALL was followed by molecular and hematologic relapses after a few months (Figure 1A). Fifteen (52%) patients were MRD positive at the post induction point; among them, 14 (93%) and 1 (7%) underwent hematologic and extramedullary relapse (in the CNS), respectively (Figure 1B).

Survival analysis showed a significant difference between these two groups, compared for event-free survival (from CR to hematologic/extramedullary relapse) (p<0.0001) (data not shown). In the MRD negative group the absence of MRD was confirmed except for the two cases with CNS relapse, described above at all the subsequent checkpoints during follow-up. No differences were observed in clinical and biological presenting features at diagnosis (age, gender, white blood cell count and immunophenotypic analysis) between patients with hematologic continuous complete remission and those who relapsed (data not shown).

Our study suggests that molecular MRD monitoring in adult ALL is a better method for identifying patients with a high or low risk of relapse than other commonly described prognostic indicators. In our experience MRD monitoring failed to predict isolated CNS relapse in 2 patients. In these