Clinical, Biologic, and Histologic Features of Late Relapses in Diffuse Large Cell Lymphoma

By Fernando Cabanillas, William S. Velasquez, Fredrick B. Hagemeister, Peter McLaughlin, and John R. Redman

After completing chemotherapy and achieving a complete remission (CR), patients with diffuse intermediate-grade lymphoma and immunoblastic lymphoma are usually considered cured if they are able to maintain that remission continuously for 24 months. Recently, we observed a number of patients with these disorders who relapsed after a continuous CR of ≥30 months from the beginning of therapy or 24 months from completing chemotherapy. This finding led us to examine 503 consecutive cases to determine the risk of late relapse and their clinical and biologic features. We found that the overall risk of late relapse of those who attained CR was 6.8%, but several features at presentation were associated with a higher risk: (1) the presence of a divergent histology; (2) a sclerosing large cell lymphoma; (3) a diagnosis based on an extranodal site with no nodal tissue available for examination. When none of these features were present, the risk of late relapse was minimal (only 3%). When any of these features was present, the risk was 14%. Most striking was the 43% late relapse rate of patients with divergent histology. All but one of the eight B-cell tumors studied at relapse showed λ light chain restriction. Five of these eight had a low S phase at the time of relapse, suggesting chemotherapeutic selection of a clone of cells with a low proliferative potential that could have given rise to the late relapse. Nucleic acid flow cytometry and immunophenotypic studies on three tumors at initial diagnosis and after relapse failed to support the hypothesis of a second de novo lymphoma and were consistent with a true recurrence of the original tumor. The results of salvage chemotherapy in this group of late relapses showed a high CR rate (57%) but no evidence of a trend for cure in the time to treatment failure curve. In contrast to the experience with Hodgkin’s disease, retreatment with the same or a similar regimen used for the original induction was not associated with durable response. Clinicians should be aware of the potential for a late relapse in cases with divergent histology and the need for new treatment strategies for such cases.

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The malignant lymphomas represent a heterogeneous group of disorders with variable clinical presentations and potential for cure. The diffuse intermediate-grade lymphomas, of which the diffuse large cell type is the most common, rank among the most curable of all these disorders. Most investigators have recognized that practically all patients with this cell type who are able to attain and maintain a complete response for 24 consecutive months are cured because late relapses seldom occur after this period of follow-up. Recently, we and others have observed several patients who have relapsed beyond this point. We undertook this study to define the quantitative risk of late relapse, the clinical and biologic features of these cases, and their outcome after salvage therapy.

MATERIALS AND METHODS

Between 1974 and 1987, 503 consecutive previously untreated cases of Ann Arbor stage I-IV diffuse intermediate-grade lymphoma or immunoblastic lymphoma were treated in our institution with combination chemotherapy regimens based on cyclophosphamide and doxorubicin with or without radiotherapy. All patients had been staged according to the Ann Arbor system. The histologic diagnosis was confirmed by a member of the Department of Pathology. Only patients with diffuse large cell, diffuse mixed, and immunoblastic lymphoma were included in this study.

After therapy, 383 (76%) patients achieved a complete remission (CR). Twenty-six complete responders who relapsed beyond 30 months of starting chemotherapy are the subject of this study. Thirty months from initiation of chemotherapy corresponds approximately to 24 months from completion of chemotherapy because it usually takes 6 months to complete treatment. Rebiopsy at relapse was accomplished in 22 of the 26 cases. Nucleic acid flow cytometry was performed on 13 cases: 10 at relapse and three at both initial diagnosis and relapse. The technique used has been described previously. Immunophenotyping was performed on seven cases at relapse using antibodies against light chains and pan-T and -B markers. In one case, bel-2 gene rearrangement studies were performed.

Salvage chemotherapy was administered to 24 patients; two patients received no salvage therapy. It is too early to evaluate their response to salvage therapy in three cases, thus leaving 21 evaluable for response. Of these 21 cases, six received retreatment with either CHOP ± Bleo (three cases) or, if no more Adriamycin was feasible, COP ± Bleo (three cases); five were treated with either cyclophosphamide or ifosfamide plus methotrexate–VP-16 combinations (methyl gap, ifosfamide, methotrexate, etoposide [MIME], 1; cytoxan, methotrexate, etoposide, dexamethasone [CMED], 4); four with an Ara-C-platinum-based regimen; and six with either MOPP, radiotherapy, or single agents.

Survival and time to treatment failure curves were plotted using the Kaplan-Meier actuarial method. The χ² method was used to test differences in the frequency of late relapses of the various histologic features.

RESULTS

Influence of histologic features at diagnosis. Table 1 summarizes the histologic features of the late relapsers at the time of their original diagnosis. The most common feature associated with late relapse was the presence of a divergent histology at the time of diagnosis. Forty-three percent of those patients experienced a late relapse in contrast to 5% for the group with pure histology (P < .01). Divergent histologies were defined as the coexistence of
diffuse large-cell lymphoma (DLCL) in one site and a low-grade lymphoma in another site. Most commonly, this occurred as DLCL in a node and small cleaved cell lymphoma (SCCL) in the bone marrow.

When the initial biopsy material was derived from an extranodal site, without the benefit of a lymph node to examine for histologic architecture, or when the diagnosis was sclerosing LCL, the frequency of late relapses was also higher (8%) than in those cases in which the diagnosis was made on a lymph node biopsy showing pure DLCL or diffuse mixed lymphoma (DMxL), in which the relapse rate was 3% (P = .07).

The 26 late relapses were analyzed separately for the presence of other features at either diagnosis or relapse (Table 2). In addition to the above histologic features, two other characteristics were observed: five cases showed a low percentage of S phase (<5%) by nucleic acid flow cytometry at relapse (only one of these five had a low-grade cell type) and one exhibited a bcl-2 rearrangement at diagnosis. Diffuse intermediate grade lymphomas usually have an S phase ranging from 6% to 15%.14 Rearrangement of bcl-2 gene is a feature more characteristic of follicular lymphomas. Only three cases (12%) of all late relapses failed to show any distinctive features.

Table 2 lists the diagnoses at the time of relapse and compares them with the histologic findings at the time of diagnosis. Of the seven cases who showed either divergent or low-grade histologies at relapse, four had divergent histologies at the time of the original diagnosis. Another case had a pure DLCL at diagnosis but exhibited a bcl-2 rearrangement, and one had sclerosing DLCL, thus leaving only one case of low-grade or divergent histology at relapse who did not show any unusual features at diagnosis.

The Ann Arbor stage of the 26 late relapses at the time of diagnosis was: I = 2, II = 6, III = 8, IV = 10.

Table 4 lists the number of late relapses according to treatment regimen used at the time of diagnosis. The lower frequency of late relapses in the CHOP-HOAP-B-IMVP-16 regimen could be related to the fact that there was only one patient in that regimen who had a divergent histology at diagnosis.

**Failure pattern.** Figure 1 illustrates the time to treatment failure (TTF) curve of all 503 cases in this study as measured from onset of therapy. The arrows show the time points at which late relapses occurred. These points ranged from 30 to 170 months. This curve includes all patients irrespective of their response to therapy.

**Tumor cell studies before and after relapse.** In three patients, fresh biopsy material was available to us at diagnosis as well as after relapse. Immunophenotyping and nucleic acid flow cytometry were performed at both of these time points and the results are summarized in Table 5. Patients no. 1 and 2 showed identical light chain phenotype and DNA content at diagnosis as well as relapse. The S phase was low for both at relapse and higher at diagnosis, suggesting that a subclone of cells with a lower S phase was responsible for the late relapse.

**Case no. 3** showed a T-cell phenotype at diagnosis and relapse characterized by CD, positivity and κ and λ negativity.

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### Table 1. Late Relapses in Diffuse Aggressive Lymphomas: Histologic Features at Time of Original Diagnosis

<table>
<thead>
<tr>
<th>Feature</th>
<th>N</th>
<th>CR</th>
<th>Late Relapse from CR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>503</td>
<td>383</td>
<td>26 (7)</td>
</tr>
<tr>
<td>Divergent histology</td>
<td>26</td>
<td>21</td>
<td>9* (43)</td>
</tr>
<tr>
<td>Extramodal biopsy</td>
<td>121</td>
<td>77</td>
<td>6 (8)</td>
</tr>
<tr>
<td>Sclerosing LCL</td>
<td>27</td>
<td>24</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Pure DLCL or DMxL</td>
<td>329</td>
<td>261</td>
<td>9 (3)</td>
</tr>
</tbody>
</table>

*The divergent histologic types were follicular small cleaved in two cases, follicular large cell in one, small cleaved cell in five (diagnosed in bone marrow when architecture not possible to determine), and small lymphocytic in one.

### Table 2. Late Relapses in Diffuse Aggressive Lymphomas: Biologic and Pathologic Features of 26 Late Relapses

<table>
<thead>
<tr>
<th>Feature</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>26</td>
<td>100</td>
</tr>
<tr>
<td>Divergent histology at diagnosis</td>
<td>9</td>
<td>35</td>
</tr>
<tr>
<td>Diagnosis based on extranodal biopsy</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>Low % S phase at relapse</td>
<td>5 /13</td>
<td>38</td>
</tr>
<tr>
<td>Sclerosing LCL at diagnosis</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>bcl-2 rearrangement at diagnosis</td>
<td>1 /1</td>
<td>11</td>
</tr>
<tr>
<td>No distinctive feature at diagnosis or relapse</td>
<td>3*</td>
<td>11*</td>
</tr>
</tbody>
</table>

*Because not all cases were studied for bcl-2 rearrangements or flow cytometry, it is possible that the member of patients with no distinctive feature at diagnosis or relapse could be less than 3% and less than 11%, respectively.

### Table 3. Histologic Features at Relapse Compared With Features at Diagnosis

<table>
<thead>
<tr>
<th>Histology at Relapse</th>
<th>N</th>
<th>Histology at Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Divergent (DLCL node, SCCL)</td>
<td>1</td>
<td>Divergent (DLCL node, SCCL)</td>
</tr>
<tr>
<td>Divergent (FLCL node, SCCL)</td>
<td>1</td>
<td>Divergent (DLCL node, FLCL)</td>
</tr>
<tr>
<td>SCCL (by fine needle aspira-</td>
<td>1</td>
<td>Divergent (DLCL node, SCCL)</td>
</tr>
<tr>
<td>tion)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCCL (in bone marrow)</td>
<td>1</td>
<td>DLCL (bcl-2 rearranged)</td>
</tr>
<tr>
<td>FMxL</td>
<td>1</td>
<td>DLCL sclerosing type</td>
</tr>
<tr>
<td>SCCL</td>
<td>1</td>
<td>DLCL, pure</td>
</tr>
<tr>
<td>DLCL</td>
<td>5</td>
<td>DLCL, pure</td>
</tr>
<tr>
<td>LCL (by fine needle aspira-</td>
<td>6</td>
<td>DLCL, pure</td>
</tr>
<tr>
<td>tion)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLCL</td>
<td>2</td>
<td>DLCL sclerosing (1), DLCL (1)</td>
</tr>
<tr>
<td>DMxL</td>
<td>2</td>
<td>DLCL, pure</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. Late Relapses According to Treatment Regimen Used

<table>
<thead>
<tr>
<th>Regimen</th>
<th>N</th>
<th>CR (%)</th>
<th>Late Relapses (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOP-Bleo</td>
<td>300</td>
<td>277 (76)</td>
<td>18 (8)</td>
<td>.12*</td>
</tr>
<tr>
<td>CHOP-Bleo/CMED</td>
<td>161</td>
<td>114 (76)</td>
<td>6 (5)</td>
<td></td>
</tr>
<tr>
<td>CHOP-HOAP-B-IMVP-16</td>
<td>52</td>
<td>42 (81)</td>
<td>0 (0)</td>
<td>.06†</td>
</tr>
</tbody>
</table>

*CHOP-Bleo late relapses versus CHOP-Bleo/CMED.
†CHOP-HOAP-Bleo-IMVP-16 versus all others.
ity. The DNA index and percentage of S phase were different at relapse when compared with the pretreatment specimen, suggesting the possibility that a de novo lymphoma was responsible for the "late relapse." However, the clinical circumstances suggested otherwise because this patient's original presentation was in the skin of the foot and leg and the relapse 30 months later was in the same region, thus suggesting a local recurrence of the same process. T-cell receptor gene rearrangements were not done.

An additional seven patients had immunophenotypic studies performed on the relapse sample but not at the time of the original diagnosis (Table 6). All but one of these seven were of B-cell phenotype and all but one of the six B-cell tumors were κ type. When considered together with cases no. 1 and 2, there were seven of eight B-cell tumors that marked as κ at the time of late relapse.

Salvage chemotherapy and survival postrelapse. Table 5 summarizes the results of salvage chemotherapy administered to 21 of the 26 patients. In two cases, no treatment was administered at relapse and it is too early for analysis in three cases. The CR rate was 57%. The salvage regimen used was selected in the most part according to the existing protocol active at the time the patient relapsed. For that reason, there were several treatment regimens used throughout this period of time. Salvage treatment regimens used were divided into four categories: retreatment with the original regimen such as CHOP or, in cases when it was thought that further Adriamycin was not feasible, COP ± Bleo was used (N = 6); an ifosfamide or cyclophosphamide plus methotrexate–VP-16–based regimen such as MIME or CMED (N = 5) ; a high-dose Ara-C/platinum regimen (N = 4); or miscellaneous treatments such as radiation alone or single agents (N = 6). Six of the 12 CRs remain in remission (three treated with Ara-C/platinum, two with MIME or CMED, and one with MOPP); all others have relapsed (Table 7).

Figure 2A illustrates the survival after relapse. The median survival for all 26 cases after relapse was 36 months, with no plateau yet evident in the curve.

Figure 2B divides the 22 cases according to the histologic diagnosis at the time of relapse. A survival advantage is seen for those cases who at the time of relapse exhibited either a divergent histology or a low-grade cell type. The outcome of those with pure aggressive histology at relapse was extremely poor, with only 40% projected to be alive at 24 months.

Figure 3 illustrates the TTF of the 12 patients who attained a CR with salvage chemotherapy. The median TTF was 28 months, but the pattern of the curve is not suggestive of a trend for cure. However, most of the patients treated with modern salvage regimens are represented in the early part of the curve.

**DISCUSSION**

In this large series of patients with diffuse aggressive lymphoma, most of whom had a diffuse large-cell type, the late relapse rate of those who attained CR was 7%. There were several histologic features at presentation that were associated with a higher risk of late relapse. The most striking of these features was the presence of a divergent histology, of whom 43% experienced a late relapse. Other such features included a diagnosis of diffuse lymphoma based exclusively on an extranodal biopsy or a sclerosing LCL. Both of these features were associated with an 8% risk of late relapse in contrast to only 3% for patients presenting with a pure diffuse lymph node architecture (Table 1). It is well known that follicular lymphomas will...
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Fig 2. Late relapses diffuse aggressive lymphomas. (A) Survival of all cases after relapse. Total, 26; dead, 14. (1) Alive. (B) Survival according to diagnosis at relapse. (a) Low grade (total, 7; dead, 1); (b) large cell (total, 15; dead, 10); (1) still in CR. P = .01.

frequently fail to manifest their follicular architecture when they invade an extranodal organ. This could explain why late relapses are more common in cases in which the biopsy was obtained exclusively from an extranodal site. Also, the diagnosis of a diffuse sclerosing LCL has been considered by many pathologists to be associated with an underlying follicular lymphoma which is not evident due to the intense sclerosis that obscures the follicular pattern.

When we compare our series with other published series, several similarities emerge. In the Dana-Farber experience using m/M-BACOD in 193 patients with stage II-IV diffuse large cell, their late relapse rate was 11 of 140 (7.8%) of complete responders. They defined late relapse as any relapse that took place after 24 months from achieving CR. This late relapse rate is remarkably similar to our own experience (26 of 383, 6.8%). The phenomenon of relapse with a low-grade histology was evident in the Dana-Farber series in which their latest relapses (61 and 72 months) had a lower-grade type at the time of recurrence. However, some differences are also evident. In the first place, there were six relapses beyond 7 years in our series (range, 80 to 170 months), while the latest relapse in the Dana-Farber series was at 7 years. This result might be due to the longer follow-up in our series, which extends to 15 years. Secondly, in their series, all but 1 of the 11 late relapses occurred in patients with Ann Arbor stage III/IV presentations, while in ours, 8 of 26 late relapses presented with Ann Arbor stage I-II. This difference might be in part due to the inclusion of stage I cases in our series, while these were not represented in the Dana-Farber series. Nevertheless, there were six stage II cases in our series of 26 late relapses, as contrasted to only 1 of 11 in the Dana-Farber series.

In the Southwest Oncology Group series, late relapses occurred up to 7 years after attaining a CR. Their longest follow-up was 12 years. Aside from this, no details are given regarding the features of late relapsers.

One of the most important questions regarding late relapses is whether they are bona fide late relapses or whether they might actually represent de novo lymphoma. To answer this question definitively, it would require that fresh tissue be available at diagnosis and relapse. In this study, there were three instances in which tissue was available for immunophenotyping as well as for nucleic acid flow cytometry studies at both time points. The results of such studies showed that in the two cases of B-cell lymphoma, the same B-cell phenotype and the same light chain restriction were observed at relapse. This finding is more suggestive of a recurrence rather than a de novo presentation, although it cannot rule out the latter possibility. In the third case, immunophenotyping could not be used to evaluate clonality because it was a T-cell lymphoma, but it is important to point out that the recurrent lymphoma proved to be also of T-cell type. Flow cytometry in cases no. 1 and 2 showed the same degree of aneuploidy at diagnosis and relapse, but both showed a lower S phase at relapse, hinting at the possibility that a subclone with a lower S phase was responsible for the recurrence. In case no. 3, the recurrent tumor was diploid, in contrast to an aneuploid DNA content at presentation. The S phase was higher at relapse. These features suggested that a different tumor might have arisen in this patient 30 months later. However, the fact that it originated in the same leg and from the same site (skin) and with the same immunophenotype suggests that it probably was a true relapse. Nevertheless, we cannot completely rule out a de novo lymphoma without gene
rearrangement studies, which unfortunately were not available in any of these cases.

It has been said in the past that patients with Hodgkin’s disease who relapse late can be reinduced and cured by using the same chemotherapy regimen they were exposed to originally.13 Of six patients managed in this fashion in our study, four achieved a second CR but all four have recurrent a second time. Even though the numbers are small, our data suggest that retreatment with the same regimen is probably not the optimal management. The high-dose Ara-C-platinum combinations appear to be active in this setting, but the follow-up is not long enough to justify firm conclusions regarding their curative potential. At this point, the TTF curve for complete responders to salvage therapy (Fig 3) does not suggest that they can be cured. Newer salvage modalities will be necessary to treat these patients with curative intent. However, the fact that many of them actually relapse with a low-grade histologic type makes it unlikely that they will be cured by current standards, particularly if their relapse is not confined to lymph nodes. It is a well-known fact that stage IV low-grade lymphomas are at present not considered to be curable by any of the front-line regimens used so far.

Finally, clinicians should be aware of the high risk for late relapse in patients presenting with divergent histologies.

REFERENCES


Clinical, biologic, and histologic features of late relapses in diffuse large cell lymphoma

F Cabanillas, WS Velasquez, FB Hagemeister, P McLaughlin and JR Redman