To the editor:

Pathogenic complexity of gastric B-cell lymphoma

We read with great interest the recent work of Starostik et al regarding gastric marginal zone B-cell lymphomas of mucosa-associated lymphoid tissue (MALT) type and their findings, suggesting 2 distinct pathogenic pathways of development for this lymphoma type.1 The findings obtained by us with a different technique and in an independent population2 are in very good agreement with their data and thus support the conclusion drawn by the authors, highlighted in the accompanying summary by Dan Longo.3 They supplement various other studies in this field,4-6 confirming our concept of at least 2, if not 3, distinct genetic subgroups.

We studied 52 extranodal B-cell lymphomas: 18 extranodal marginal zone B-cell lymphomas of MALT type (MZBL-MTs), 7 MZBL-MTs of the gastrointestinal tract with a diffuse large B-cell component (giMZBLplusLBCLs), and 27 diffuse large B-cell lymphomas of the gastrointestinal tract without small cell component (giLBCLs) using comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH). The translocation t(11;18) was found as the sole aberration in 2 MZBL-MTs only, favoring the view that this translocation blocks this lymphoma progression from the small to the large cell component.7-9 In contrast, t(11;18)-negative MZBL-MTs were characterized by frequent gains on chromosome 3 and DNA amplifications on 2p13-p15, including the REL proto-oncogene. Furthermore, we found a clonal lymphoma progression from the small to the large cell component with accumulation of gains and losses of chromosomal material in the large-cell component in giMZBLplusLBCLs. Aberrations overlapping with MZBL-MTs and giMZBLplusLBCLs included losses on chromosome 13, amplifications of the REL proto-oncogene or gains on chromosome 12. Additionally, the large-cell component revealed gains on 8q24, including amplifications of the MYC proto-oncogene, and losses on 2q. The giLBCLs had frequent gains on chromosomes 12, 9, and 11q and losses on 6q.10 We concluded that, based on the distinctive and partly overlapping patterns of genetic aberrations, MALT lymphomas can be divided into different genetic subgroups (Figure 1). First, MZBL-MTs may be divided according to presence or absence of t(11;18). t(11;18)-positive cases have no further detectable aberrations and are therefore characterized by high biological stability. Second, t(11;18)-negative MZBL-MTs have a characteristic pattern of gains and losses with frequent gains on chromosome 3. But the presence of overlapping aberrations such as amplifications of the REL proto-oncogene, losses on 13q, and the identical IgH rearrangement of the small- and large-cell component reflect clonal evolution of MZBL-MTs toward the LBCLs. Third, a subset of giMZBLplusLBCLs shows a high frequency of gains on 8q24, including amplifications of the MYC proto-oncogene and losses on 2q. This suggests a different line of lymphomagenesis and progression for at least some of these lymphomas, since these aberrations are not present in giLBCLs and suggest the presence of diffuse large B-cell lymphoma arising de novo. Furthermore, a recent report further fueled discussion by showing that biclonal gastric lymphomas exist as true composite stomach lymphomas,11 Therefore, the pathogenic pathways leading to gastric lymphomas are likely to be even more complex than suggested by Starostik et al.

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Figure 1. Hypothetical grouping of mucosa-associated B-cell lymphomas on the basis of molecular cytogenetics. “+” refers to gains and “−” to losses of chromosomal material on the given chromosomal region; “+++” refers to aberrations significantly more frequent in giMZBLplusLBCL. (Figure slightly modified from Barth et al.2 ©2001 Wiley-Liss Inc, a subsidiary of John Wiley & Sons. Used by permission.)
The pathogenesis of extranodal gastric marginal zone B-cell lymphoma of MALT type (eMZBCL) and its aggressive counterpart, extranodal gastric diffuse large B-cell lymphoma (eDLBCL), is a jigsaw puzzle composed of many pieces: genetic aberrations occurring during lymphomagenesis. Taking the simplest approach, using classical cytogenetic analysis our group identified a translocation, t(11;18)(q21;q21), whose presence or absence divides the eMZBCLs into two groups: t(11;18)-positive ones and t(11;18)-negative ones. Already at that time, it was noticed that the eDLBCLs did not display this translocation. These findings were confirmed by others later.

In a recent paper, Barth and colleagues agree with these concepts of fundamental difference in the pathogenesis of eMZBCLs and eDLBCLs and offer a hypothesis on the further subgrouping of the t(11;18)-negative tumors. Although they have only 2 t(11;18)-positive lymphomas in their series and the frequency of aberrations they are able to detect is in fundamental difference in the pathogenesis of eMZBCLs and eDLBCLs. We identified a translocation, t(11;18)(q21;q21), whose presence or absence divides the eMZBCLs into two groups: t(11;18)-positive ones and t(11;18)-negative ones. Already at that time, it was noticed that the eDLBCLs did not display this translocation. These findings were confirmed by others later. 

Recently, work is in progress to clarify the importance of 6q aberrations in the eMZBCLs versus eDLBCLs and the fact that we were able to identify 2 groups of DLBCLs characterized by the t(11;18) translocation from a pre-existing eMZBCL showing the same aberration. The 6q aberration displaying cases might be primary eDLBCLs. In contrast, the t(11;18)-negative eMZBCLs are characterized by increased frequency of genetic aberrations. The spectrum of aberrations identified by both groups (by different methods) in the t(11;18)-negative eMZBCLs is quite similar (aberrations on chromosomes 3, 11, and 18). In the high-grade eDLBCLs, with the exception of gains on 8q24 and 9q, the results for chromosomes evaluated by Barth et al are analogous. Particularly, if one compares studies performed using the same technique: the comparative genomic hybridization (CGH) study of Barth et al with a previously published CGH study by Peters et al, the latter performed on exactly the same material we used for microsatellite analysis.

But one must remain cautious regarding the grouping of the t(11;18)-negative tumors, as depicted in Figure 1 of Barth et al’s preceding letter. Aberrations displayed here are puzzle pieces that have not found their proper places yet: they were just recognized to belong to the game. Some of the alterations in the proposed groups, such as gains on chromosomes 7 in the eMZBCL or 18 in the eDLBCL, have been each identified in one case only, raising questions regarding the significance of these findings. Other frequent aberrations from our work (losses of the p53 and APC gene loci) not detected by CGH due to the shortcomings of this method were not considered in this scheme at all. Moreover, the analyzed material was heterogeneous; only 50% of the eMZBCLs were tumors of gastric origin. This contrasts with our work, in which only gastric lymphomas were analyzed. Differences in origin are known to be reflected in the frequency of the t(11;18) in these lymphomas and could lead also to different genetic aberrations suffered during lymphomagenesis. Thus, conclusions based on the data obtained by Barth et al should be called a hypothesis awaiting a confirmation.

Our data also support the role of aberrations on chromosomes 3, 11, and 18 in the development of (secondary?) eDLBCL. Deletions of 5q21 (APC), 9p21(INK4A/ARF), 13q14 (RB), and 17p13 (p53) seem to make the difference between eMZBCLs and eDLBCLs, as we detected them in the latter only. Most interesting, however, is the striking difference in the frequency of 6q aberrations in the eMZBCLs versus eDLBCLs and the fact that we were able to identify 2 groups of DLBCLs characterized by the t(11;18) translocation from a pre-existing eMZBCL showing the same aberration. The 6q aberration displaying cases might be primary eDLBCLs. Currently, work is in progress to clarify the importance of 6q deletions in primary high-grade eDLBCL and in tumors containing both eMZBCLs and eDLBCL components. We will be happy to cooperate in the further characterization of these tumors with other groups, especially if they have suitable material available.

We agree completely that pathogenetic pathways in gastric B-cell lymphoma are a puzzle that is still pretty far from being solved. The scheme will not be simple, but we must start from a common denominator. We must keep in mind that the aberrations described in both works occurred at the genomic DNA level. It will be a major step to proceed from the rather insensitive method of DNA screening to the identification of the relevant candidate genes and the evaluation of their role in the lymphomagenesis of gastric B-cell lymphoma.

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References


Response:

An emerging concept of diverse pathogenetic pathways in gastric B-cell lymphoma
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References

To the editor:

Do B-cell chronic lymphocytic leukemia patients with Ig V\textsubscript{H}3-21 genes constitute a new subset of chronic lymphocytic leukemia?

With interest we read the report of Tobin et al\textsuperscript{1} describing a new subset of B-cell chronic lymphocytic leukemia (B-CLL). After mutation analysis of rearranged immunoglobulin variable heavy chain (IgV\textsubscript{H}) genes, they observed that B-CLL patients with somatically mutated V\textsubscript{H}3-21 genes have an unfavorable clinical course similar to B-CLL patients with unmutated immunoglobulin genes.

Their presented data and conclusions need some comments. In several studies,\textsuperscript{2-4} as well as in our own unpublished data, a preferential usage of several V\textsubscript{H} genes is observed. Data of a selection of frequently used V\textsubscript{H} genes are summarized in Table 1. In all studies V\textsubscript{H}1-3 is highly represented in B-CLL and is almost exclusively unmutated. But the frequent occurrence of the V\textsubscript{H}3-21 gene found by Tobin et al, namely in 13% of all CLL, could not be confirmed by the 3 other studies indicated (Table 1; studies 2-4).

This discrepancy of usage of the V\textsubscript{H}3-21 gene is statistically significant ($\chi^2$ analysis for V\textsubscript{H}3-21 usage: studies 1 versus 2, $P = 0.013$; studies 1 versus 3, $P = 0.008$; studies 1 versus 4, $P = 0.020$). In addition, a statistically significant difference is observed for the overall mutation frequency between studies 1 and 2 (Table 1; $\chi^2$ analysis, $P = 0.004$).

Several explanations for these different findings can be proposed: there might be a wrong annotation due to closely related germline IgV\textsubscript{H} genes. V\textsubscript{H}3-48 is the immunoglobulin gene with the highest homology to the V\textsubscript{H}3-21 gene, namely, 10 basepair differences between both genes at nucleotide level; but it seems unlikely that, due to mutations at most of these positions, the germline gene is not correctly assigned. Or there might be methodologic differences between the different studies. Each of the studies inevitably excluded samples that failed on the IgV\textsubscript{H} mutation analysis, which is interesting information since this might give some insight in the sensitivity of the assays to detect clonal rearrangements. Finally, there might be a different patient population treated at each center or a particular patient group selected for IgV\textsubscript{H} sequencing.

Next to the difference in usage of the V\textsubscript{H}3-21 gene, the relatively low mutation load observed in the somatically mutated cases described by Tobin et al is striking. Thirteen patients showed a sequence homology to the germline V\textsubscript{H}3-21 gene between 92.8% and 98%. Ten of those show a homology between 96% and 98% (Table 1, study 1). The threshold value of 98% to distinguish unmutated from mutated IgV\textsubscript{H} genes was originally chosen because polymorphisms, which are quite common in V\textsubscript{H} genes, can account for 2% of disparity.\textsuperscript{4} But an alternative use of a 96% threshold is currently under debate. Kröber et al,\textsuperscript{5} analyzing 300

Table 1. The most frequent V\textsubscript{H} genes used in unmutated and mutated B-CLL across 4 studies

<table>
<thead>
<tr>
<th>Homology to germinel</th>
<th>Unmutated/mutated cases, by study*</th>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
<th>No. 4</th>
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<tbody>
<tr>
<td>V\textsubscript{H}1-69</td>
<td>Below 98%</td>
<td>21/0</td>
<td>8/2</td>
<td>5/1</td>
<td>9/1</td>
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<td>V\textsubscript{H}3-07</td>
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<td>0/4</td>
<td>0/5</td>
<td>1/8</td>
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<td>Below 98%</td>
<td>0/3</td>
<td>0/7</td>
<td>2/1</td>
<td>3/8</td>
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<tr>
<td>V\textsubscript{H}3-23</td>
<td>Below 98%</td>
<td>0/3</td>
<td>0/7</td>
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<tr>
<td>V\textsubscript{H}3-30</td>
<td>Below 98%</td>
<td>0/3</td>
<td>0/7</td>
<td>2/0</td>
<td>6/4</td>
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<tr>
<td>V\textsubscript{H}3-30</td>
<td>Below 98%</td>
<td>0/3</td>
<td>0/7</td>
<td>2/0</td>
<td>6/4</td>
</tr>
<tr>
<td>Total number of patients</td>
<td>Overall frequency of cases, %</td>
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<td>74</td>
<td>64</td>
<td>84</td>
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<tr>
<td>Unmutated/mutated cases, %</td>
<td>Below 98%</td>
<td>58/42</td>
<td>35/65</td>
<td>48/52</td>
<td>45/55</td>
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<tr>
<td>Below 98%</td>
<td>NM</td>
<td>47/53</td>
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<td>Forward primer(s)</td>
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<td>V\textsubscript{\textsuperscript{H}L1} to V\textsubscript{\textsuperscript{H}L6}</td>
<td>V\textsubscript{\textsuperscript{H}L1} to V\textsubscript{\textsuperscript{H}L6}</td>
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<tr>
<td>Reverse primers(s)</td>
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<td>J\textsubscript{H}</td>
<td>J\textsubscript{H}</td>
<td>J\textsubscript{H}</td>
<td></td>
</tr>
</tbody>
</table>

NM indicates not mentioned.

*Study 1 is Tobin et al; study 2, AZ Sint-Jan, Brugge, unpublished data, 2002; study 3, Fais et al; study 4, Hamblin et al.

†Aubin et al.
Pathogenic complexity of gastric B-cell lymphoma

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