Long-term persistence of molecular disease after histological remission in low-grade gastric MALT lymphoma treated with *H. pylori* eradication. Lack of association with translocation t(11;18): a 10-year updated follow-up of a prospective study


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Background: Localized low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma can regress after *Helicobacter pylori* eradication, but IgVH gene monoclonality may persist. We studied the long-term histological and molecular follow-up of 24 patients and the possible association of t(11;18) with the persistent monoclonality.

Patients and methods: From January 1994, 24 untreated patients with stage I low-grade gastric MALT lymphoma associated with *H. pylori* were prospectively studied. They all received eradication treatment and were sequentially followed-up with endoscopies for histological and molecular studies. Rearrangement of the IgVH gene was studied by PCR analysis. MALT1 locus alterations were studied by FISH.

Results: Twenty-two of the 24 patients (91%) achieved disappearance of the lymphoma. Eighteen (82%) of the 22 histologically cured patients and 16 of the 19 (84%) with long follow-up had monoclonality. Three patterns of development of IgVH gene rearrangements were observed: four patients (21%) had polyclonal rearrangements; eight (58%) had maintained/intermittent monoclonality and four (21%) had occasional monoclonality, mostly after *H. pylori* reinfection. Only one patient (6%) with persistent monoclonality relapsed. The remaining 18 patients maintained the remission, despite the persistent monoclonality in 15, for a median of 66 months (range 20–113). t(11;18) was not found in any of the patients with persistent monoclonality. Time and the number of endoscopies performed were not related with the occurrence of monoclonality.

Conclusions: In stage I low-grade gastric MALT lymphoma eradication of *H. pylori* achieves prolonged histological remission in 90% of patients, but molecular remission is not accomplished in most cases. Molecular disease persists for years, but is not associated with t(11;18).

Key words: lymphoma, MALT, MALT lymphoma, gastric lymphoma, *H. pylori*, t(11;18)

Introduction

It is now well established that *Helicobacter pylori* induces the apparition of lymphoid tissue in the stomach as follicular gastritis constituting the mucosa-associated lymphoid tissue (MALT) and is also related to the development of lymphomas in this specialized lymphoid tissue [1–4]. The strongest evidence for this association was the demonstration of the histological regression of the lymphoma after the eradication of *H. pylori* [4]. After the initial report of Wotherspoon et al. [4] other studies confirmed that 55%–93% of patients with low-grade gastric MALT lymphoma in stage I can be histologically cured after *H. pylori* eradication [5–9]. Also, in the series that had a long enough follow-up, the responses seemed to be maintained for a long time, with only a small proportion, about 10%, of the patients relapsing [9–13]. These facts brought up two additional issues. The first was the identification of the factors that might influence the response of MALT lymphoma to the eradication of *H. pylori*. Now it is clear that the presence of a high-grade component in the lymphoma [6] and the penetration of the lymphoma deep through

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the gastric layers to serosa or proximal lymph nodes are the more important structural factors [6, 14, 15]. The presence of molecular abnormalities in the lymphoma also has crucial prognostic influence. The translocation t(11;18)(q21;q21) that fuses the MALT1 gene on chromosome 18 with the API2 gene on chromosome 11 is the most important molecular abnormality detected in MALT lymphomas [16, 17] and gastric MALT lymphoma determines a peculiar clinical behaviour, with extensive disease [18] and absence of response to antibiotic eradication treatment [19–21]. The second issue was the long-term persistence of monoclonal IgV\textsubscript{H} gene rearrangement in patients with histological regression of the lymphoma. This persistent monoclonality has been demonstrated in half of the histologically cured patients but, despite the many cases observed, its prognostic significance is not yet clear [9–13, 22].

This paper presents the updated follow-up of a prospective series of patients with stage I gastric MALT lymphoma studied since 1994 [8, 11]. We report the histological and molecular response to treatment and, although cases had been accrued over those years, we can now also report the long-term outcome of the initial cases that have been followed-up for as long as 10 years. Specific aims have been to study the outcome of the monoclonality after this long-term follow-up and its possible association with the presence of t(11;18)(q21;q21).

Patients and methods

In December 1993 we began a prospective study to evaluate the eradication of \textit{H. pylori} as initial therapy in patients with stage I low-grade gastric MALT lymphoma. All consecutive untreated patients associated with \textit{H. pylori} newly diagnosed at the Hospital Ramon y Cajal (Madrid, Spain) were included in the study. The initial results of this series from 1994 to 1996 [8] and thereafter to December 2000 have been previously reported elsewhere [11]. The present reappraisal of the series refers to the 24 patients included from December 1993 to December 2002. Only patients with a follow-up of more than 18 months up to July 2004 have been included in the present report. All included patients were white, born in Spain of Spanish ancestry.

Full details of the study procedure have been previously reported [8, 11]. Low-grade MALT lymphoma diagnosis was established by histological criteria and active association with \textit{H. pylori} demonstrated in the biopsy samples and with a positive urea \textsuperscript{13}C breath test. All patients underwent complete staging to confirm stage I of the Lugano system [23], accepted their inclusion in the study and received standard eradication triple therapy administered for 14 days. Patients were followed-up with clinical assessment and sequential endoscopic examinations 2–3 months after eradication treatment, then, ideally, every 3 months the first year, every 6 months the second year and thereafter once a year. In the sequential endoscopies, mapped biopsies were obtained for \textit{H. pylori} culture and for histological and molecular studies. Histological evaluation of the response followed the system described by Wotherpoon et al. [4]. Complete histological regression (CHR) was only accepted when no definitive lymphoma was histologically demonstrated and confirmed in at least two subsequent endoscopies. A polymerase chain reaction (PCR) analysis of the IgV\textsubscript{H} gene was performed using semi-nested procedures with consensus primers for the V\textsubscript{H} region (Fr\textsubscript{3A}) in conjunction with nested primers directed to the J\textsubscript{H} region (LJH and VLJH) as described elsewhere (8, 11). MALT1 rearrangement indicating the presence of t(11;18)(q21;q21) was investigated by double color FISH assay in the same samples where the diagnosis of MALT lymphoma had been performed. For this purpose, the original blocks of all cases were retrieved from the archive. Material adequate for this study or a definitive result was obtained in only 13 patients. There was no selection bias in performing the study in any special subset of the patients. For detection of alterations affecting the MALT1 locus the commercially available LSI-MALT1 probe (Vysis, Downers Grove, IL) was applied on diagnostic paraffin-embedded tissue sections. Briefly, deparaffinized sections were incubated in 0.05 mg/ml pepsin pH 2 at 37°C for 30 min, washed with 2 × SSC for 5 min, fixed in 10% buffered formalin for 5 min and dehydrated in graded ethanol. Probe and target DNA were simultaneously denatured at 83°C for 3 min and incubated overnight at 37°C. Post-hybridization washes were performed with 2 × SSC/0.3% NP-40 for 2 min at 75°C. Sections were counterstained and mounted with DAPI/antifade (Q-BIO gene).

Statistical analysis

In order to evaluate whether the probability of detecting monoclonality was related to the timing or to the increasing number of endoscopies performed during the follow-up, linear regression analyses were performed. For the former, the proportion of patients with monoclonality in every sequential endoscopy after CHR (proportion of patient with monoclonality at the first endoscopy, at the second endoscopy, etc) was evaluated. As endoscopies were not necessarily performed at the same point during the follow-up, for each patient every endoscopy was numbered (first, second, and so on) and clonality information obtained at each endoscopy compared. This allowed analysis of the proportion of patients with monoclonality versus time. For the latter, the analysis evaluated the proportion of monoclonality in each individual patient in the sequential endoscopies performed during the follow-up after CHR (frequency of the test analysis).

Results

Of the 24 patients, 11 were male (46%) and 13 were female (54%). The mean age was 57 years (range 26–76 years). \textit{Helicobacter pylori} was eradicated in all 24 patients after the initial course of antibiotic treatment. Of the 24 patients, 22 (91%) achieved a histological disappearance of the gastric MALT lymphoma after \textit{H. pylori} eradication, which occurred a mean of 5.3 months (range 2–19 months) after the treatment. Nineteen patients (86%) achieved a regression of the lymphoma between 2 and 8 months after treatment (mean 3.7 months, range 2–8), but in patients 15, 16 and 21, a consistent histological regression of the lymphoma was achieved only after 19, 11 and 15 months, respectively. The histological and molecular data during the follow-up of the 22 patients achieving a CHR are shown in Table 1 and Figure 1.

In the 22 patients achieving CHR, the remission was maintained for a mean of 51 months (range 20–112). Only one patient (patient 16) (4.5%) relapsed after 25 months and was treated with chlorambucil achieving a CHR maintained for 36 months. Two patients were lost to follow-up after 2 and 8 months and another patient died due to a gastric carcinoma 6 months after eradication treatment and while in remission of the lymphoma. The 19 remaining patients in CHR were in CHR for a mean of 64 months (range
Table 1. Low-grade gastric MALT lymphoma in stage I treated with *H. pylori* eradication. MALT1 gene rearrangement, histological response to eradication, IgV\textsubscript{H} gene rearrangement and outcome

<table>
<thead>
<tr>
<th>MALT1-R</th>
<th>Response</th>
<th>PCR</th>
<th>Pattern</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>1</td>
<td>Neg</td>
<td>NR</td>
<td>M</td>
<td>Surgery, CHR for 10 years</td>
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<tr>
<td>2</td>
<td>CR</td>
<td>M(i)</td>
<td>2</td>
<td>CHR for 112 months</td>
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<tr>
<td>3</td>
<td>Neg</td>
<td>CR</td>
<td>M(i)</td>
<td>CHR for 110 months</td>
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<tr>
<td>4</td>
<td>CR</td>
<td>M(o), 104 months</td>
<td>3</td>
<td>CHR for 104 months</td>
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<tr>
<td>5</td>
<td>CR</td>
<td>M</td>
<td></td>
<td>Lost after 2 months</td>
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<tr>
<td>6</td>
<td>Neg</td>
<td>CR</td>
<td>M(o), 80 months</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>CR</td>
<td>P</td>
<td></td>
<td>Lost after 8 months</td>
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<tr>
<td>8</td>
<td>CR</td>
<td>M → P</td>
<td>1</td>
<td>CHR for 113 months. Reinfection</td>
</tr>
<tr>
<td>9</td>
<td>CR</td>
<td>M(i)</td>
<td>2</td>
<td>CHR for 98 months. Reinfection (M)</td>
</tr>
<tr>
<td>10</td>
<td>Neg</td>
<td>CR</td>
<td>M(i)</td>
<td>CHR for 88</td>
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<tr>
<td>11</td>
<td>CR</td>
<td>M</td>
<td></td>
<td>Dead of carcinoma after 6 months</td>
</tr>
<tr>
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<td>CR</td>
<td>P</td>
<td>1</td>
<td>CHR for 39 months</td>
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<td>CR</td>
<td>M(i)</td>
<td>CHR for 75 months</td>
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<td>14</td>
<td>CR</td>
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<td>CHR for 57 months</td>
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<tr>
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<td>Neg</td>
<td>CR</td>
<td>M(i)</td>
<td>CHR for 62 months</td>
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<tr>
<td>16</td>
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<td>CR</td>
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<td>Relapse at 25 months</td>
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<td>CR</td>
<td>M(i)</td>
<td>CHR for 55 months</td>
</tr>
<tr>
<td>18</td>
<td>Neg</td>
<td>CR</td>
<td>M (o) 55 months</td>
<td>3</td>
</tr>
<tr>
<td>19</td>
<td>CR</td>
<td>M(o) 24 months</td>
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<td>22</td>
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<td>NR</td>
<td>M</td>
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<td>CR</td>
<td>M(i)</td>
<td>CHR for 30 months</td>
</tr>
<tr>
<td>24</td>
<td>Neg</td>
<td>CR</td>
<td>M(i)</td>
<td>CHR for 20 months</td>
</tr>
</tbody>
</table>

MALT1-R, MALT1 gene rearrangement (FISH); Neg, negative; CHR, complete histological remission; NR, no remission; PCR, IgV\textsubscript{H} rearrangement; M, monoclonal; M(i), monoclonal intermittent; M(o): monoclonal occasional; P, polyclonal. Reinfection (M), *H. pylori* reinfection (and monoclonality).

Pattern: monoclonality pattern (1, polyclonal; 2, monoclonal persistent/intermittent; 3, monoclonal occasional).

Figure 1. Histological and molecular development of the 19 followed-up patients who achieved complete histological regression of the lymphoma after *H. pylori* eradication (see text). ◊ denotes polyclonal rearrangement of the IgV\textsubscript{H} gene; ○ denotes monoclonal rearrangement of the IgV\textsubscript{H} gene. Numbers refer to the histological score as described by Wotherspoon et al. [4]: score 0, normal; 1, chronic active gastritis; 2, chronic active gastritis with follicle formation; 3, suspicious lymphoid infiltrate in lamina propria, probably reactive; 4, suspicious lymphoid infiltrate in lamina propria, probably lymphoma; 5, LG MALT lymphoma. Numbers in isolation (outside the symbols) correspond to biopsies with histological study (histological score) but no molecular study. R: Patient 16 relapsed after 25 months.
20–113). Excluding patient 16 who relapsed, the remaining 18 patients had been in stable histological remission for a mean of 66 months (range 20–113). The 10 followed-up patients included in the study between 1994 and the end of 1997 had been in complete remission for a mean of 89 months (39–113 months).

The two patients (patients 1 and 22) without response and active lymphoma had persistent monoclonal IgVH gene rearrangement. Of the 22 patients achieving a CHR, 18 (82%) had monoclonality after the histological disappearance of the lymphoma in at least one of the biopsies obtained during the follow-up. Excluding the two patients lost to follow-up and the patient who died of gastric carcinoma, 19 patients had long-term follow-up. Monoclonality was found in 16 of the 19 (84%). Only three of the 19 patients (15.7%) had sustained polyclonal rearrangement (patients 12, 20 and 21) and another one (patient 8) had monoclonality and histological response in the first biopsy and thereafter steady polyclonality. Altogether four patients (21%) had stable polyclonal rearrangements either from the CHR or after conversion from early monoclonality (pattern 1). Eleven patients (58%) had a maintained or intermittent monoclonality (pattern 2). In four patients (21%), monoclonality was occasionally found in biopsies performed at 104 (patient 4), 80 (case 6), 55 (patient 18) and 24 (patient 19) months after treatment, while the rest of the samples (before and after) showed a polyclonal rearrangement (pattern 3). Only one of the 15 patients with persistent/intermittent or occasional monoclonality (6.6%) had a lymphoma relapse (Table 1 and Figure 1).

The linear regression analysis showed that there was no significant correlation between the proportion of patients with monoclonality in the biopsies obtained and the timing of subsequent endoscopies after achieving CHR ($\beta = -0.01, P = 0.324$), suggesting that monoclonality was not dependant on the time lapse after CHR. There was no correlation either between the proportion of monoclonality demonstrated and the number of endoscopies in each individual patient ($\beta = -0.02, P = 0.367$) suggesting that the occurrence of monoclonality was not dependant on the number of endoscopies performed.

*Helicobacter pylori* reinfection was demonstrated in four patients (patients 6, 8, 9 and 18) 80, 113, 18 and 55 months after the initial eradication (Table 1). Along with the reinfection, monoclonality was detected in three patients (75%); in two of them (patients 6 and 18) this was the only moment in which monoclonality was detected, in all other samples the IgVH gene rearrangement was polyclonal (pattern 3). The third patient (patient 9) already had persistent/intermittent monoclonality (pattern 2). Despite the reinfection, the lymphoma did not relapse in any of these patients and in all four a further antibiotic treatment successfully eradicated *H. pylori*.

MALT1 rearrangement was not demonstrated in any of the 13 patients studied in this series. Therefore, neither the two patients who did not respond to eradication therapy, nor the relapsed patient, nor the patients with persistent/intermittent or occasional monoclonality showed MALT1 locus alteration.

**Discussion**

A high proportion of histological responses can be expected in patients with low-grade gastric MALT lymphoma in stage I after *H. pylori* eradication. When the patients had a long follow-up, the long-term evaluation of the response could also be assessed. The original six cases of Wotherspoon et al. were in CHR after 6 years of follow-up [22]. Thiede et al. [10] observed that 77 of 97 patients (79%) achieved a response that was maintained for a median of 33 months, with only eight relapses observed (10%) and Fischbach et al. [13] observed that 56 of 88 patients (62%) maintained CHR after a mean follow-up of 44 months, with 8% of relapses. In the present series, 22 of 24 (91.6%) patients achieved a CHR of the lymphoma and the responding patients maintained the response after a mean of 64 months, with only one patient (4.5%) having relapsed. Moreover, patients treated between 1994 and 1996 have been in remission for 8–10 years, the longest follow-up reported to date.

The other issue that we addressed in this study was the persistent monoclonality of the IgVH gene after CHR of the MALT lymphoma. This persistent monoclonality was observed in the pioneer publication by Wotherspoon et al. in 1993 [4, 22] and was found in 30%–70% of patients in other studies [7–12]. In our initial report of nine patients studied between 1994 and 1995 and followed-up for a median of 14 months, eight (89%) achieved a CHR and 50% had monoclonality, which appeared to be transforming to polyclonality [8]. In the subsequent study [11], including 19 patients with a mean follow-up of 37 months, 18 (94%) achieved a CHR, but monoclonality was observed to be maintained or intermittently present in 11 (61%). In the present study including 24 patients with a median follow-up of 64 months, the response proportion was maintained (91%), but monoclonality was detected during follow-up in 18 of the 22 patients (82%) who had achieved a CHR and in 16 of the 19 (84%) who had prolonged follow-up. When in our initial observation the monoclonality seemed to disappear with time [8], the original hypothesis was that cure of the lymphoma seemed to follow an orderly sequence: first, disappearance of the macroscopic lesions at endoscopy, and then histologically detectable lymphoma with only persisting monoclonality, which also tended to disappear. When the patients in this series were followed for a longer time, this was the case only in the minority (21%) showing pattern 1, suggesting a molecular remission of the lymphoma. Eleven patients (58%) had either a persistent or intermittent monoclonality (pattern 2) that could be demonstrated for years, indicating the persistence of the lymphoma. In addition to these two patterns, when the follow-up is as prolonged as in our study, a new pattern (pattern 3) emerged. There are some patients that can be considered as in pattern 1 for years, but unexpectedly monoclonality might have been demonstrated as late as 80 and 104 months. It can be argued that it is the same situation as in pattern 2 described above, but the possibility can be also considered that this monoclonality represents the reappearance of a latent lymphoma population. In most cases the detection of occasional
monoclonality coincided with *H. pylori* reinfection suggesting that this might be a triggering event and that monoclonality might be the first detectable step of reactivation of a latent lymphoma. This finding is in agreement with the fact that *H. pylori* reinfection has been previously demonstrated to be related with lymphoma relapses [13, 24].

Pattern 1 and pattern 2 have also been recognized by Thiede et al. [10], considering that the first might represent a molecular cure of the lymphoma, whereas in the second the monoclonality had been demonstrated to be derived from a lymphoid population that is identical to the original lymphoma, actually representing a minimal residual disease. Our data indicate that when the patients have a prolonged follow-up, pattern 1 is infrequent and that in the majority, a pattern 2 or pattern 3 develops, with no tendency towards a molecular remission of the disease, although this latent lymphoma has not conditioned clinical relapses so far. This situation is often found in other lymphoproliferative diseases and a tentative parallel model might be follicular lymphoma, which after therapy may achieve and maintain a clinical complete remission, despite the persistence in bone marrow or peripheral blood of a PCR-detected clonal residual lymphoma population bearing bcl-2/IgH rearrangements [25, 26].

The linear regression analysis showed that monoclonality does not seem to be dependant on the time lapsed from CHR (since the proportion of patients with monoclonality was not different in the sequential endoscopies) or on the number of endoscopies performed (as the proportion of monoclonality in each patient is similar in the subsequent endoscopies). However, these data should be interpreted with care, as the influence of artefacts in the sampling of the biopsies might have played a decisive role and cannot be disregarded.

Considering that t(11;18) has a crucial influence on the histological response of gastric MALT lymphoma to eradication, as positive patients do not respond or have a rapid relapse whereas most of the patients that do respond do not have the chromosomal abnormality [19, 20, 21, 27], we have studied the possible influence of t(11;18) in the persistence of monoclonality. We have demonstrated no relation, since t(11;18) was not demonstrated in any of the patients with any pattern of persistent monoclonality. In our laboratory we have studied t(11;18) in 18 patients with low-grade gastric MALT lymphoma (13 current patients and five others), which was detected only in two patients (11%) not included in this study, both with disseminated disease. Therefore, lack of MALT1 locus rearrangements cannot be attributed to technical problems since we have definite positive cases. The low frequency in our series is in contrast with other studies, where it has been found in about 30%–40% of patients. In the initial report by Liu et al. [19], t(11;18) was demonstrated in nine of 22 patients (41%) and in a further large international study it was found in 44 of 111 patients (39%) from a collaborative group (GELD) and eight institutions from five different European countries [20]. In a large study including 417 patients from the UK, France and Germany [28], frequencies varied greatly among different sites of the MALT lymphoma, being highest in the small intestine (69%) and lung (38%), and lower than has previously been considered in the stomach (24%).

All these data seem to have been obtained from patients coming from different cities and countries and possibly of different racial origins, whereas our data were obtained from white patients born in Spain of Spanish ancestry in a single institution and probably reflect the frequency of t(11;18) in our environment. To our knowledge there is no other data on the frequency of t(11;18) in localized gastric low-grade MALT lymphoma in Spain and currently this low proportion seems to represent the real frequency. A similar low frequency also seems to be the case when other local populations are studied: t(11;18) was detected in none of the 18 patients with limited gastric lymphoma responding to eradication in a German trial reported by Alpen et al. [27] and in another study from Japan it was found in only two of 24 (8.3%) patients with gastric MALT lymphoma [29].

The use of different techniques may partially explain the differences in frequency in between our series and others. In most series, analysis of t(11;18) has been performed by RT-PCR detecting API2-MALT1 fusion transcript. This technique may have more sensibility for detecting the translocation than FISH on paraffin sections. As t(11;18) has been significantly associated with Cag-A positive strains of *H. pylori* [30], it is also possible that the low frequency in our environment depends on geographical differences conditioning different strains of *H. pylori*. Also considering that t(11;18) negative cases might have a better response to the eradication therapy, the good outcome of our series might have been influenced by the absence of the chromosomal alteration.

In conclusion, in our environment eradication of *H. pylori* can achieve histological remission in 90% of patients with low-grade gastric MALT lymphoma, which can be maintained for years. Despite this favorable response, most patients do not achieve molecular remission of the lymphoma and have distinct patterns of persistent monoclonality of the IgVH gene. Persistent molecular lymphoma is not associated with t(11;18) and so far (after 10 years of follow-up) has not conditioned clinical relapses. *Helicobacter pylori* reinfection, at least in some patients, might have a role in the occurrence of occasional monoclonality.

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