Molecular response assessed by PCR is the most important factor predicting failure-free survival in indolent follicular lymphoma: Update of the MDACC series

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Summary

Background: We have observed that molecular response, as defined by a PCR-negative status during the first year of therapy, along with β2-microglobulin (β2M), was the most important variable associated with failure-free survival (FFS) in follicular lymphoma (FL). Herein, we present an update of the previously published MDACC series.

Patients and methods: A total of 116 patients (male:female ratio 64:52; median age: 52 years) with indolent FL and BCL-2 rearrangement (at MBR or mcr breakpoints) assessable in peripheral blood (pb) by PCR prior to treatment, and with two or more PCR determinations during the first year, were selected for the present study.

Results: Of the 116 patients, 4 who presented with progression and 1 who died of unrelated causes during the first year were excluded from the landmark analysis. One hundred patients (86%) achieved clinical CR and 80 (69%) achieved a negative PCR status within first year. Median FFS was 6.4 years. Five-year FFS was 73% and 28% for molecular responders and nonresponders, respectively (P = 0.001). In spite of this strikingly higher FFS favoring molecular responders, no clear-cut plateau was evident in this group. Molecular response assessed in pb (P = 0.001) and serum β2M (P < 0.001) were the most important factors to predict FFS in the multivariate analysis. In the subset of patients with normal β2M and molecular CR, there was a trend for a plateau in the FFS curve. No significant difference between the groups has been observed so far in terms of survival.

Conclusions: Molecular response assessed in pb using a PCR technique is, along with β2M, the most important factor to predict FFS in FL.

Key words: BCL-2, follicular lymphoma, minimal residual disease, PCR, prognostic factors

Introduction

Although patients with follicular lymphoma (FL) often achieve a clinical complete response (CR), the majority of them, particularly those with advanced presentations, eventually relapse [1, 2]. This probably occurs because residual lymphoma persists below the detection threshold of standard procedures. FLs are characterized in up to 85% of the cases by the t(14;18) translocation, resulting in the rearrangement of the BCL-2 oncogene [3]. The BCL-2/IgH rearrangement can be used as a marker to detect minimal residual disease by means of a polymerase chain reaction (PCR) technique [4]. Using this method, we and others have been able to show persistent cells carrying t(14;18) after therapy in FL patients [4-11]. More recently, we carried out a longitudinal study to analyze the molecular response, defined as achievement of a PCR-negative status, in a large series of previously untreated patients [12]. A molecular response, assessed in peripheral blood (pb), occurred in close to one half of the patients. Moreover, molecular response during the first year of treatment together with the initial serum β2-microglobulin (β2M) levels were the most important factors associated with failure-free survival (FFS) in these patients with indolent FL [12].

The aim of the present paper is to update the above-mentioned series with a considerably longer follow-up in order to confirm our previous observation related to the importance of molecular response in patients with FL.

Patients and methods

Using a PCR assay, we previously analyzed the pre-treatment pb of a large series of patients with FL for the presence of BCL-2 gene rearrangements [12]. For the outcome analysis, we selected 116 patients (64 male/52 female; median age 52 years) with a BCL-2 rearrangement (at either the 'major breakpoint region' (MBR) or the 'minor cluster region' (mcr)), demonstrated prior to treatment and in whom two or more PCR determinations were available during the first year of therapy. This subset of 116 patients is the subject of the present update evaluation. The distribution with regard to the BCL-2/IgH breakpoint site was as follows: MBR site, 102 cases (88%); mcr site, 14 cases (12%).
was defined as the disappearance of all signs and symptoms of disease. Maintenance was used after completion of chemotherapy in most cases (34%), and grade 3 in 3 cases (3%). Only three patients with grade 3 histology whose tumor predominantly contained cleaved cells presented, it was determined that serum LDH and (52M levels, chest X-ray, CT scans of thorax, abdomen, and pelvis, and bilateral bone marrow biopsies. The histologic distribution was follicular center cell lymphoma grade 1 in 73 cases (63%), grade 2 in 40 cases (34%), and grade 3 in 3 cases (3%). Only three patients with grade 3 histology whose tumor predominantly contained cleaved cells were considered indolent and admitted into the study as grade 3 histology. Twenty-three patients presented with stage I-II, 19 with stage III, and 74 with stage IV. Patients were treated according to the stage with combination chemotherapies and radiotherapy as previously detailed [12]. Overall, 67 patients received ATT (alternating triple therapy) [13], whereas 31 received the combination FND (fludarabine, mitoxantrone (novantrone), and dexamethasone). Interferon maintenance was used after completion of chemotherapy in most cases. Follow-up consisted of physical examination, routine blood tests, and CT scans. If positive at diagnosis, bilateral bone marrow biopsies were repeated after every three to four cycles during the first year and every four to six months thereafter. Complete remission (CR) was defined as the disappearance of all signs and symptoms of disease as determined by clinical, radiographic, and laboratory parameters. Partial remission (PR) was defined as a reduction of 50% or more in measurable disease for at least one month. Any other responses, including mixed response, stable disease, progressive disease, early death, or death from toxicity, were considered treatment failures.

Samples from both peripheral blood and/or bone marrow aspirates were collected before starting therapy, whenever possible every three to four months for the first two years after starting therapy, and then every six months until relapse or progression of the lymphoma. The detailed PCR methods used have been previously described [4]. Molecular response was defined as the disappearance of (14;18) amplicons in peripheral blood at any given point during or after therapy in a patient with a known baseline BCL-2 rearrangement. Molecular response during the first year was considered as the achievement of a PCR-negative status at any point during the first year of treatment.

Failure-free survival (FFS) was measured from the start of therapy until relapse or toxic death. Patients not relapsing were censored at the last follow-up. Actuarial probability of FFS was estimated according to the method of Kaplan-Meier, and curves compared using the log-rank test. The association of molecular response to FFS was evaluated by two approaches: 1) the landmark method, comparing the FFS after one year with regard to the molecular response at that time, and 2) the use of a proportional hazards regression model.

Results

After one year of therapy, clinical CR was attained in 103 patients (88%) and PR in 13 (12%). Forty-three patients have so far developed progression, with an actuarial FFS of 86% at two years (95% confidence interval (95% CI): 79%-93%) and 60% at five years (95% CI: 49%-71%) (Figure 1).

As previously described, the molecular response rates progressively increased during the first year of therapy, with 82 patients (72%) reaching a molecular response in pb during this time. No pre-treatment variable predicted the achievement of a molecular response. No correlation was observed between treatment regimen and molecular response.

In Figure 2, FFS is compared one year after the start of therapy with the molecular response status during the first year. Only patients who achieved a molecular response at any point during the first year were considered molecular responders. There was a substantial failure-free advantage for patients with evidence of molecular response within the first year of therapy (five-year FFS: 73% (95% CI: 62-84) vs. 28% (95% CI: 7-49); P < 0.001). No plateau was observed in either responder or nonresponder subsets. The same landmark analysis was done only in patients who achieved clinical CR during the first year (103 cases out of 116). The substantial difference in FFS between molecular responders and nonresponders was preserved (P = 0.01), suggesting a further prognostic role for molecular response beyond that of clinical response.

In a separate analysis of the association between the main pre-treatment characteristics and FFS (results not presented), it was determined that serum LDH and β2M values were the two factors most closely associated with FFS. The second approach to analyzing the association between molecular response and FFS involved fitting a proportional hazards model with a time-dependent covariate indicating whether a molecular response had
achieved with standard-dose chemotherapy and b) We also showed that a) molecular remissions can be bone marrow of more than 80% of these cases [12].

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Discussion
The t(14;18) translocation provides an excellent target which can be applied to the detection of minimal residual disease in peripheral blood and bone marrow. Using the PCR technique, we detected t(14;18) in the blood and/or bone marrow of more than 80% of these cases [12]. We also showed that a) molecular remissions can be achieved with standard-dose chemotherapy and b) molecular remissions correlate with durable clinical remissions. In fact, the most important conclusion of the previous study [12] was that the molecular response at different time points during the first year of therapy strongly correlates with failure-free survival, with more than 3/4 of the patients who achieved a PCR-negative state at any of the tested time points (between 3 and 14 months) being alive and in clinical CR five years after starting therapy. In the updated series, the differences in outcome between responders and nonresponders within the first year of treatment are still very important. However, there is not a clear-cut plateau for either responder or nonresponder subsets. This indicates that, in spite of molecular response, most patients with FL eventually progress. Our results are similar to those reported by Gribben et al. [8, 10, 14] in a different patient population: after high-dose therapy and autologous stem-cell transplantation, those patients who became PCR-negative experienced a better FFS than those who did not. Such results have been confirmed in other series after transplantation [15, 16].

We also found that both the molecular response within the first year and the pre-treatment serum β2M were independent and important predictive factors for FFS. The combination of β2M and molecular response allows us to identify a group of patients whose prognosis is excellent, with approximately 75% of them projected to be alive and in CR five years later (Figure 3). This group with excellent prognosis represents approximately one half of all the FL cases in this study. Indeed, longer follow-up will be necessary to determine if a significant proportion of those patients are cured. On the other hand, a small subset of patients consisting of approximately 15% of the population are at high risk of early relapse. These cases should be considered candidates for investigational therapy in the future.

Data presented herein refer to molecular response assessed only in pb. The molecular analysis of material obtained in bone marrow could also be important to better assess a real 'molecular complete response'. In fact, for Gribben et al. [17] such a source of DNA was apparently more sensitive than pb to assess residual disease and, subsequently, to predict FFS. In our experience [12], pb PCR seemed to be almost as sensitive as bone marrow and, from a clinical standpoint, is easier to obtain.

In conclusion, the use of serial PCR analysis to determine the molecular response is a very useful tool, especially when combined with other prognostic factors such as β2M.

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References


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