Anti-idiotype antibody response after vaccination correlates with better overall survival in follicular lymphoma

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Running title: OS and immune response through idiotype vaccine
Abstract

Previous studies demonstrated that vaccination-induced tumor-specific immune response is associated with superior clinical outcome in follicular lymphoma patients. Here, we investigated whether this positive correlation extends to overall survival (OS). We analyzed 91 untreated patients who received CVP chemotherapy followed by idiotype vaccination. Idiotype proteins were produced either by the hybridoma method or by expression of recombinant idiotype-encoding sequences in mammalian or plant-based expression systems. We found that achieving a CR/CRu to CVP and making an anti-Idiotype antibody are two independent factors that each correlated with longer OS at 10 years (90% vs 68% with or without a CR/CRu, p = 0.024; 90% vs 69% with or without tumor-specific antibody production, p = 0.027). In the subset of patients received hybridoma-generated vaccines, we found that anti-Idiotype production was even more highly associated with superior OS (p=0.0016), and this was the case even in patients with a PR to CVP (p=0.0008).

Introduction

Targeted immunotherapy with Rituximab represents one of the most exciting advancement in treatment of follicular lymphoma, contributing to improved overall survival (OS) in this disease over the past two decades. Another attractive target for immunotherapy is immunoglobulin idiotype (Id), a clonal marker expressed on tumor B cells. Previous studies have shown that the induction of
tumor-specific immune responses through Id vaccination has been associated with superior clinical outcome \(^7\text{--}^{14}\). In accordance with previous observations, a phase III randomized trial, evaluating progression free survival (PFS) in patients received customized idiotype versus non-specific vaccines, demonstrated longer PFS in patients who generated anti-Id antibody response \(^{15}\). In this study, with mature followup data, we investigated whether the positive correlation of immune response and PFS extends to OS.

**Patients, material and methods**

**Patient characteristics and idiotype vaccination**

This retrospective study included 91 newly diagnosed follicular lymphoma patients who had given written consent in accordance with the Declaration of Helsinki to be treated on IRB- approved idiotype vaccine protocols at Stanford University between 1979 and 2000.

The clinical characteristics of the patients are described in Supplemental Table 1. FLIPI scores were available on 84 out of 91 patients with the distribution of 19\% low, 58\% intermediate and 23\% high. This group of patients had very favorable outcome with OS of 77\% and 62\% at 10 and 15 years, respectively.

All 91 patients first received CVP chemotherapy as initially described by Bagley et al. (cyclophosphamide 400 mg/m\(^2\) PO days 1-5, vincristine 2 mg day 1, prednisone 100 mg/m\(^2\) days 1-5) for 2 cycles beyond the best response \(^{16}\). All patients were evaluated for treatment response (CR, CRu, PR, SD, PD) by the
criteria of Cheson et al. 17. Sixty patients achieved clinical and radiographic CR/CRu, all but 4 repeated bone marrow biopsy at the completion of chemotherapy. These 4 patients, along with 13, who were found to have residual disease in the bone marrow despite of radiographic CR/CRu, were scored partial responders (CR/CRu=43). After cytoreduction, patients received vaccination at least 2 months after completion of chemotherapy as reported previously 7-9, 18.

Anti-idiotype immune response
The detection of anti-idiotype (anti-Id) humoral and cellular responses after vaccination was carried out by ELISA and T cell proliferation assays, respectively, as described previously 7. The methods and the cut-offs of immune assays were unchanged over the years, allowing us to compare historical immune response data across trials.

Statistical methods
The OS was examined by the Kaplan-Meier method (PRISM, GraphPad software, San Diego, CA). Cox proportional hazards model was used in the univariate and multivariate analyses.

Results
Response to CVP and overall survival
All 91 patients received CVP as induction chemotherapy, which yielded 43 (47%) CR (35) or CRu (8), 44 (48%) PR and 4 (4%) SD/PD. We found that patients
who achieved CR/CRu after CVP had significantly longer OS than those who did not. At 10 years, the OS was 89% for patients with CR/CRu, and 68% for patients without (p = 0.024, Figure 1A).

**Induction of anti-idiotype immune response and overall survival**

The induction of anti-Id humoral and cellular immune responses was evaluated. Thirty-three patients (36%) produced antibodies against their own idiotype and not against unrelated idiotypes (specific antibody response, sAb). Fourteen patients (15%) produced antibodies that recognized their own idiotype as well as one or two unrelated idiotypes (poly-specific antibody response, pAb). Specific anti-Id T cell response was seen in 23 patients (26%), 25 patients (28%) produced poly-specific T cell response.

Induction of specific anti-Id antibody via vaccination was associated with superior OS (Figure 1B, p = 0.029). At 10 years, the OS was 90% and 69% for patients with or without specific anti-Id antibody induction, respectively. Historically, we have reported results only in relation to specific antibody response. Interestingly, production of poly-specific anti-Id antibody appeared to be beneficial as well (Figure 1C). Thus, we combined specific and poly-specific immune responses in our subsequent analysis. We showed that generation of antibody response was correlated with a longer OS (Figure 1D); in contrast, generation of T cell response was not (data not shown).

Over the years, the methods of producing idiotype vaccines have evolved from hybridoma rescue technology to molecular cloning-based technology\textsuperscript{15,18,19}. 
Here, we explored the outcome of vaccination according to the methods of vaccine production. The current study included patients received vaccines produced by the hybridoma method (n = 61), and the method of expressing idiotype-encoding sequences in mammalian cells (n = 16) and in tobacco plants (n = 14). We found that the proportion of the patients generating anti-Id antibodies was not significantly different in each vaccine group, although anti-Id T cell response rate appeared to be higher in the molecular cloning groups (data not shown). In addition, we observed that the correlation between anti-Id antibody response and OS was particularly pronounced in the hybridoma group, even in patients who only had a PR to CVP (Fig. 1E and 1F). In this study, we did not find the association between tumor-specific T cell response and favorable clinical outcome, which has been demonstrated by other investigators. This may be due to the variability of T cell assays and the lack of the “gold standard” to measure tumor-specific T cell response, as demonstrated by Inoges et al.

We then examined whether immune response is an independent prognostic factor for survival by performing univariate and multivariate analyses. As shown in Table 1, only factors significantly associated with better OS were CR/CRu to chemotherapy and induction of anti-Id antibody, which were independent of each other, as demonstrated by the multivariate analysis.

The current study is the largest to report a correlation between immune response and superior OS in follicular lymphoma patients. The OS outcome in this group of patients was extremely favorable compared to other reported series.
This may have been due to selection bias, effective chemotherapy, or the result of idiotype vaccination. To address these questions definitively, three randomized phase III trials have been conducted. These trials differed in induction treatment, vaccine production methods and required level of cytoreduction prior to vaccination. Recently, results of two of these trials were reported, showing no significant difference in PFS between patients who received idiotype vaccine and non-specific vaccine. Only one of the reported trials collected immune response data, which demonstrated significantly longer PFS in those patients generated anti-Id antibody response, echoing our previous observation. This result raised the question whether the ability of mounting immune response after idiotype vaccination simply represents a biomarker for disease virulence or host immune competence. If so, we could attempt to identify those patients prior to vaccination who are likely to generate immune response. To this end, we investigated clinical and immunological factors that may correlate with the likelihood of anti-Id production. We found that FLIPI scores, response to chemotherapy, the percentage of T cell subsets in tumor biopsy specimens measured by flow cytometry did not impact the likelihood of generating anti-Id immune response (data not shown). Moreover, all patients were immune competent because they were able to mount immune response to the carrier protein KLH. However, KLH may be too immunogenic to reflect the subtle differences in immune competence of the host. Results from this study highlighted the importance of following OS in the randomized vaccine trials.
Authorship:

W.Z.A performed the research, analyzed the data and wrote the paper. R.T. performed statistical analysis. B.T. generated idiotype vaccines. D.C performed immune assays. R.L. designed the clinical trials, performed the research, analyzed the data and wrote the paper. The authors declare no conflict of interest.

References:

5. Hiddemann W, Kneba M, Dreyling M, et al. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin,
vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood. 2005;106:3725-3732.


Table 1 Univariate and Multivariate Analyses

A. Univariate Analysis

<table>
<thead>
<tr>
<th></th>
<th>Relative benefit for survival</th>
<th>Confidence interval</th>
<th>P value</th>
</tr>
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<tr>
<td>CR/CRu after CVP</td>
<td></td>
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<tr>
<td>CR vs non-CR</td>
<td>3.32</td>
<td>1.1, 10.2</td>
<td>0.033</td>
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<td>Anti-Id antibody response</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>negative vs positive</td>
<td>0.355</td>
<td>0.132, 0.957</td>
<td>0.041</td>
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<tr>
<td>Anti-Id cellular response</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>negative vs positive</td>
<td>1.09</td>
<td>0.42, 2.85</td>
<td>0.86</td>
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<td>Age at diagnosis</td>
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<td></td>
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<td>&lt; 60 yo vs &gt; 60 yo</td>
<td>1.02</td>
<td>0.977, 1.07</td>
<td>0.37</td>
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<td>FLIPI</td>
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<td>low vs high</td>
<td>1.65</td>
<td>0.274, 9.96</td>
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<td>intermediate vs high</td>
<td>1.97</td>
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B. Multivariate Analysis

<table>
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<td>3.28</td>
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<td>Anti-Id antibody response</td>
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<td>negative vs positive</td>
<td>0.360</td>
<td>0.132, 0.988</td>
<td>0.047</td>
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</table>
Figure Legends

Figure 1. Kaplan-Meier estimates of overall survival according to anti-Id immune response. (A) OS according to response to CVP chemotherapy. CR/CRu (solid line): n = 43. < CR/CRu (dashed line): included patients who achieved a partial response, stable disease or progressive disease, n = 48. (B) OS in patients with (n = 33, solid line) or without (n = 58, dash line) specific anti-Idiotype antibody production. (C) OS in patients who generated specific (n = 33, solid line), polyspecific (n = 14, dotted line) or no anti-Id antibody (n = 44, dash line). (D) OS in patients with (n = 47, solid line) and without (n = 44, dash line) anti-Id antibody response. (E) and (F) Kaplan-Meier estimates of OS in patients who received hybridoma-produced idiotype vaccines. (E) OS according to anti-Id antibody response. Ab pos (solid line): n = 33, Ab neg (dash line): n = 28. (F) OS according to anti-Id antibody response in patients who achieved a PR to cytoreduction chemotherapy (n = 33) after CVP chemotherapy. Ab pos (solid line): n = 16. Ab neg (dash line): n = 17. sAb pos and sAb neg: with or without generating specific anti-Id response. pAb: polyspecific anti-Id response. Ab pos: patients who generated either specific or polyspecific anti-Id antibody response. Ab neg: patients who did not generate anti-Id antibody response. Definitions of specific and polyspecific antibody responses are described in the text.
Figure 1

A

B

C

D

E

F
Anti-idiotype antibody response after vaccination correlates with better overall survival in follicular lymphoma

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