Brief report

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

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Previous studies demonstrated that vaccination-induced tumor-specific immune response is associated with superior clinical outcome in patients with follicular lymphoma. Here, we investigated whether this positive correlation extends to overall survival (OS). We analyzed 91 untreated patients who received CVP chemotherapy (cyclophosphamide, vincristine, and prednisone) 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 complete response/complete response unconfirmed (CR/CRu) to CVP and making an anti-idiotype antibody are 2 independent factors that each correlated with longer OS at 10 years (89% vs 68% with or without a CR/CRu, \( P = .024 \); 90% vs 69% with or without tumor-specific antibody production; \( P = .027 \)). In the subset of patients who received hybridoma-generated vaccines, we found that anti-idiotype production was even more highly associated with superior OS (\( P < .002 \)); this was the case even in patients with a partial response (PR) to CVP (\( P < .001 \)). (Blood. 2009;113:5743-5746)

Introduction

Targeted immunotherapy with rituximab represents one of the most exciting advancements in the treatment of follicular lymphoma, contributing to improved overall survival (OS) in this disease during the past 2 decades.1-6 Another attractive target for immunotherapy is the clonal immunoglobulin (idiotype, Id) 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.7-14 In accordance with previous observations, a phase 3 randomized trial in which the authors evaluated progression-free survival (PFS) in patients who received customized idiotype versus nonspecific vaccines demonstrated longer PFS in patients who generated an anti-Id antibody response.15 In this study, with mature follow-up data, we investigated whether the positive correlation of immune response and PFS extends to OS.

Methods

Patient characteristics and Id vaccination

This retrospective study included 91 patients who were newly diagnosed with follicular lymphoma and who had given written consent in accordance with the Declaration of Helsinki to be treated with Institutional Review Board–approved idiotype vaccine protocols at Stanford University between 1979 and 2000. The clinical characteristics of the patients are described in Table S1 (available on the Blood website; see the Supplemental Materials link at the top of the online article). Follicular Lymphoma International Prognostic Index (FLIPI) scores were available for 84 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/m² by mouth days 1-5, vincristine 2 mg day 1, prednisone 100 mg/m² days 1-5) for 2 cycles beyond the best response.16 All patients were evaluated for treatment response (complete response [CR], complete response unconfirmed [CRu], partial response [PR], stable disease [SD], progressive disease [PD]) by the criteria of Cheson et al.17 Sixty patients achieved clinical and radiographic CR/CRu; all but 4 had a 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.7,18

Anti-Id immune response

The detection of anti-Id humoral and cellular responses after vaccination was performed by enzyme-linked immunosorbent assay (ELISA) and T-cell proliferation assays, respectively, as described previously.7 The methods and the cutoffs 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 using Prism software (GraphPad Software, San Diego, CA). The 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 (n = 35) or CRu (n = 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 = .024; Figure 1A).

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

The induction of anti-Id humoral and cellular immune responses was evaluated. Thirty-six patients (39.5%) produced antibodies against their own Id and not against unrelated Ids (specific antibody response, or sAb). Fourteen patients (15%) produced antibodies that recognized their own Id as well as 1 or 2 unrelated idiotypes (polyspecific antibody [pAb] response). Specific anti-Id T-cell response was observed in 23 patients (26%), and 25 patients (28%) produced a polyspecific T-cell response.

The induction of specific anti-Id antibody via vaccination was associated with superior OS (P = .029, Figure 1B). 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, the production of the polyspecific anti-Id antibody appeared to be beneficial as well (Figure 1C). Thus, we combined specific and polyspecific immune responses in our subsequent analysis. We showed that the 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 Id vaccines have evolved from hybridoma rescue technology to molecular cloning-based technology. Here, we explored the outcome of vaccination according to the methods of vaccine production. The current study included patients who received vaccines produced by the hybridoma method (n = 61) and the method of expressing Id-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 the rate of anti-Id T-cell response appeared to be greater 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 (Figure 1E,F). In this study, we did not find a positive correlation between tumor-specific T-cell response and favorable clinical outcome, although such positive correlation has been demonstrated by other investigators.\textsuperscript{11} This may be attributable to the variability of T-cell assays and the lack of a “gold standard” to measure tumor-specific T-cell response, as demonstrated by Inogés et al.\textsuperscript{11}

We then examined whether immune response is an independent prognostic factor for survival by performing univariate and multivariate analyses. As shown in Table 1, the 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 treated with Id vaccination. The OS outcome in this group of patients was extremely favorable compared with other reported series.\textsuperscript{21-23} This finding may have been attributable to selection bias, effective chemotherapy, or the result of Id vaccination. To address these questions definitively, 3 randomized phase 3 trials have been conducted. These trials differed in induction treatment, vaccine production methods, and required level of cyto redemption before vaccination. Recently, results of 2 of these trials were reported and showed no significant difference in PFS between patients who received the Id vaccine versus a nonspecific vaccine.\textsuperscript{15,24} Only 1 of the reported trials collected immune response data, which demonstrated significantly longer PFS in those patients who generated anti-Id antibody response, confirming our previous observation.\textsuperscript{10,15}

This result raised the question of whether the ability of mounting immune response after Id vaccination simply represents a biomarker for disease virulence or host immune competence. If so, we could attempt, before vaccination, to identify those patients who are likely to generate immune response. To this end, we investigated clinical and immunologic 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 change 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.

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Authorship

Contribution: 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; and R.L. designed the clinical trials, performed the research, analyzed the data, and wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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