Humoral Immune Response and Immunoglobulin G Fc Receptor Genotype are Associated with Better Clinical Outcome Following Idiotype Vaccination in Follicular Lymphoma Patients Regardless of their Response to Induction Chemotherapy.

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ABSTRACT

We have reported that anti-idiotype antibody response and FcγRIIIa 158 Valine/Valine (V/V) genotype both correlate with better outcome in a group of 136 follicular lymphoma patients receiving idiotype vaccination after induction chemotherapy. Here, we examined whether this correlation is related in any way to the chemotherapy response. In CR patients, the 5-year progression free survival (PFS) was 69% for patients with antibody response and/or V/V genotype while only 40% for patients with neither, median time to progression (TTP) 10.47 vs 3.46 years (p=0.012). In PR patients, the 5-year PFS was 57% for patients with antibody response and/or V/V genotype and 17% for patients with neither, median TTP not reached vs 1.31 years (p=0.0001). This study further confirms the strong association of clinical outcome with antibody response and with the functionally more active form of the Fc receptor in patients receiving idiotype vaccination regardless their response to induction chemotherapy.
INTRODUCTION

Active immunotherapies that generate long-lasting immune responses against tumor antigen(s) by the patient may be an appealing strategy to treat non-Hodgkin's lymphoma. One approach being tested is vaccination against the unique sequences of immunoglobulin (idiotype, Id) expressed by each patient's tumor\textsuperscript{1}. The goal of vaccination is to generate anti-Id immune responses, which may actively eliminate tumor cells and lead to better outcome\textsuperscript{2}. Lymphoma patients vaccinated with Id protein make humoral and cellular anti-Id immune responses to a variable degree, depending upon the vaccine regimen\textsuperscript{3-5}. In one study, anti-Id cellular immune responses are believed to induce molecular remission in vaccinated patients\textsuperscript{6}. In contrast, we have recently shown that anti-Id humoral immune responses and FcγRIIIa 158 V/V genotype are associated with better clinical outcome in a larger group of lymphoma patients\textsuperscript{7}. In these studies, patients received Id vaccines when they were in remission after induction chemotherapy. Therefore, the response to induction chemotherapy could have had an impact on their outcome. In this report, we determined the influence of chemotherapy response on the clinical outcome and whether the predictive value for better outcome of antibody response and V/V genotype applied to patients who had different chemotherapy responses.
METHODS

Idiotype Vaccination Studies

This retrospective study included 136 patients, who received idiotype vaccination using different study protocols between 1988 and 2000. The patient characteristics were summarized in Supplemental Table 1. To be included in vaccine trials, all patients were required to receive induction chemotherapy to achieve at least a partial response before vaccination. The responses were scored according to the Cheson criteria\textsuperscript{8}. The follicular lymphoma international prognostic index (FLIPI) score at the time of induction chemotherapy was available on 127 patients to determine their risk group as described\textsuperscript{9}.

Vaccinations were initiated at least 2 months after completion of chemotherapy. During the vaccination, 86 patients received chemical adjuvant, 18 patients received GM-CSF and 32 patients had Id protein-pulsed dendritic cells\textsuperscript{1,5,10,11}. The vaccination usually composed of 4-5 monthly injections according to individual protocols. Post-vaccination follow-up was conducted every 3-4 months for the first 2-3 years, and then semi-annually or annually. The median follow-up after induction chemotherapy was 8.26 years for the entire group. All vaccination studies were conducted according to institutional review board-approved protocols and informed consent was obtained from all patients.

Immune Response Assessments
A specific humoral anti-Id immune response was determined by pre-specified criteria when a 4-fold increase in anti-Id antibody titer was found after vaccination compared to before vaccination and to the irrelevant Id proteins using enzyme-linked immunosorbent assay. Anti-Id cellular immune response was determined by T-cell proliferation assays by culturing PBMC in media alone or with tumor Id, or irrelevant Id proteins. Pre-specified criteria required that incorporation of [3H]-thymidine more than twice the background (media alone) was observed on ≥ 2 occasions to be considered positive. Immune responses were measured before vaccination, 2 weeks following each vaccination, and 3 and 6 months after last injection. In general, anti-Id immune responses were detected after 3-4 vaccinations and peaked after 4-5 vaccinations. The anti-Id antibodies were sustained for several months. The cellular immune responses declined quickly after last vaccine and were undetected after 3 months in most of the cases.

### Analysis of FcγRIIIa and FcγRIIa Polymorphisms

Genomic DNA was prepared from tumor cells or PBMC using a QIAGEN DNA extraction kit or from the serum as described. The FcγRIIIa 158 V/F and FcγRIIa 131 H/R genotypes were determined using TaqMan technology on an ABI Prism 7900HT Sequence Detector System with FcγRIIIa and FcγRIIa-specific primer pairs and allele-specific probes.

### Statistical Analysis

The median TTP and difference in the PFS were determined using the Kaplan-Meier estimation and log-rank statistic (PRISM for Macintosh, GraphPad Software, San Diego, CA). A multivariate analysis using
Cox Proportional Hazard Model was performed to identify independent prognostic variables influencing the PFS (StatView 5.0.1, SAS Inc, Cary, NC).
RESULTS AND DISCUSSION

Responses to Induction Chemotherapy and Clinical Outcome

The anti-Id immune response has been shown to correlate with better clinical outcome in follicular lymphoma patients who received a custom Id vaccine\textsuperscript{5,10}. However, these patients all received induction chemotherapy of different regimens to reduce tumor burdens and all were in remission at the time of vaccination (Supplemental Table 2). We first analyzed the influence of chemotherapy response on clinical outcome of a group of 136 consecutive vaccinated patients from our previous report, for whom even longer follow-up is now available. Overall, 93 (68\%) patients achieved CR and 43 (32\%) patients achieved PR after chemotherapy. The development of anti-Id humoral or cellular immune response and the Fc\(\gamma\)RIIIa and IIa genotype distribution did not differ between CR and PR patients (Supplemental Table 1). As expected, the CR patients had longer TTP after the last chemotherapy. The estimated 5-year PFS was 54\% for CR and 34\% for PR patients, with median TTP estimate at 5.87 and 1.62 years for two groups, respectively (\(P =0.012\)) (Figure 1a). Certain chemotherapy reagents such as fludarabine have been to shown to suppress T cell function\textsuperscript{13} and might have a negative effect on idiotype vaccination. However, in our study, use of fludarabine or adriamycin had no effect on the clinical outcome (Table 1).

The Clinical Impact of Anti-Id Abs and V/V Genotype in Different Chemotherapy Response Groups.

We have previously shown that anti-Id antibody response and/or V/V genotype correlated with longer TTP\textsuperscript{7}. This result suggested the importance of vaccine-induced anti-tumor antibodies and that these
antibodies mediate tumor killing more efficiently with effector cells bearing the FcγRIIIa of V/V genotype. Here, we analyzed the correlation of anti-Id Abs and V/V genotype together with clinical outcome in different chemotherapy response subgroups. The TTP of CR patients with anti-Id Abs and/or V/V genotype (total of 45 patients) was longer than CR patients with neither (10.47 vs 3.46 years, \( P =0.012 \)), with estimated 5-year PFS of 69% and 40% for the two groups, respectively (Figure 1b).

Similarly, PR patients with anti-Id Abs and/or V/V genotype (total of 19 patients) also had a longer TTP (not reached vs 1.31 years, \( P =0.0001 \)), with 5-year PFS of 57% and 17% for the two groups, respectively (Figure 1c). The Observations in both animal models and early clinical studies suggest that vaccines may be more effective in the setting of minimal residual disease\(^{14} \). Therefore, it was predicted that clinical impact of Id vaccination would have been more pronounced in CR patients\(^6 \). In contrast to the prediction, prolonged TTP associated with anti-Id Abs and/or V/V genotype was clearly shown in both CR and PR patients (Table 1b and 1c). In fact, the prolonged TTP was even more profound in PR patients. The PR patients who had anti-Id Abs and/or V/V genotype had an identical survival curve compared to CR patients with anti-Id Abs and/or V/V genotype (TTP: not reached vs 10.47 years, \( p=0.899 \)). In contrast, for those who had neither, PR did significantly worse than CR patients (TTP: 1.31 vs 3.46 years, \( p=0.004 \)) (Figure 1d). This suggested that antibody response may be able to control the gross residual disease and delay tumor progression in PR patients. It is the first time that a potential benefit of idiotype vaccination has been implicated in a large number of patients with gross disease. In contrast, the anti-Id cellular immune response had no impact on TTP in either CR or PR patients (data not shown). In fact, patients with cellular immune response showed a trend toward a worse outcome (Table 1). The biological explanation for this observation is not clear. One possibility is that our assay measured CD4\(^+ \) T cell response but did not measure the potentially anti-tumor cytotoxic CD8\(^+ \) T cells.
The FLIPI score were available on 127 patients. Patients of different risk groups did not differ in their chemotherapy response nor in their anti-Id immune responses (Supplemental Table 3). However, low risk patients had longer TTP than intermediate/high risk patients (Supplemental Figure 1a). Additionally, in the intermediate/high risk patients, anti-Id Abs and/or V/V genotype was associated with a longer TTP (10.47 vs 2.18 years, p<0.0001), while no difference was found within the low risk patients (Supplemental Figures 1b and 1c). This result further confirmed the association of better outcome with anti-Id Abs and/or V/V genotype in patients with unfavorable clinical features. In a multivariate analysis, CR along with anti-Id Abs and FcγRIIIa 158 V/V genotype emerged as three independent positive predictors for longer PFS, whereas none of the others had an impact (Table 1).

Our data supports a model in which anti-tumor antibody-mediated ADCC plays an important role in the anti-tumor effect of Id vaccination in both CR and PR patients. Therefore, in contrast to previous assumptions, idiotype vaccination should not be limited to patients with minimal residual disease. While some patients may have gross diseases after induction, the vaccine-induced antibody response may be able to control the disease and delay tumor progression and the need for subsequent treatment. None of the patients in this report received rituximab during their pre-vaccination cytoreduction. The clinical effect of Id vaccination may differ in rituximab-treated patients.15
REFERENCES


### TABLE 1. Prognostic Factors for Freedom from Progression: Cox Proportional Hazard Model

<table>
<thead>
<tr>
<th>Factor</th>
<th>Relative Benefit*</th>
<th>CI</th>
<th>P#</th>
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</thead>
<tbody>
<tr>
<td>158 V/V</td>
<td>4.41</td>
<td>1.90 - 10.25</td>
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<tr>
<td>131 H/H</td>
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<td>0.41 - 1.13</td>
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<td>Humoral Immune Response</td>
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<td>1.57 - 4.42</td>
<td>0.0002</td>
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<tr>
<td>Cellular Immune Response</td>
<td>0.61</td>
<td>0.35 - 1.05</td>
<td>0.075</td>
</tr>
<tr>
<td>CR</td>
<td>2.23</td>
<td>1.36 - 3.64</td>
<td>0.001</td>
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<td>Use of Adriamycin</td>
<td>0.67</td>
<td>0.37 - 1.21</td>
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<tr>
<td>Use of Fludarabine</td>
<td>1.27</td>
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<td>Stage IV</td>
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<td>0.40 - 1.18</td>
<td>0.177</td>
</tr>
<tr>
<td>Clinical Symptoms‡</td>
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<td>0.63 - 2.86</td>
<td>0.453</td>
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<tr>
<td>Age</td>
<td>0.98</td>
<td>0.96 - 1.01</td>
<td>0.182</td>
</tr>
<tr>
<td>Time from Diagnosis to Induction Chemo</td>
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<td>1.00 - 1.01</td>
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<td>GM-CSF Adjuvant</td>
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<td>0.33 - 1.35</td>
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<td>High/Intermediate FLIPI #</td>
<td>0.45</td>
<td>0.19 - 1.02</td>
<td>0.060</td>
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</table>

* Relative Benefit: to have longer freedom from progression from last chemotherapy, CI: 95% confidence intervals
# All P values are two-sided and considered to be statistically significant for P<0.05
‡ Clinical Symptoms: fever, night sweats, weight loss
# Analyzed on 127 patients with available FLIPI score
FIGURE LEGENDS

Figure 1  Kaplan-Meier estimates of progression free survival by response to induction chemotherapy, and humoral immune response and V/V genotype.

Progression free survival curves were plotted by response to induction chemotherapy (a), or by humoral anti-Id immune responses and/or FcγRIIIa 158 V/V genotype in CR (b), PR (c) and all (d) patients. CR represents patients with complete response or complete response unconfirmed. PR represents patients with partial response. Other represents patients without either anti-Id antibodies or FcγRIIIa 158 V/V genotype. TTP: median time to progression.
A.

- CR: TTP 5.87 yr
- PR: TTP 1.62 yr (p=0.012)

Progression Free Survival (%)

Years
B.

CR Humoral or V/V

CR Other

TTP

10.47 yr

3.46 yr (p=0.012)
C.

- PR Humoral or V/V
- PR Other

TTP
not reached
1.31 yr \( (p=0.0001) \)
D.

- CR Humoral or V/V: 10.47 yr
- PR Humoral or V/V: not reached
- CR Other: 3.46 yr
- PR Other: 1.31 yr

Progression Free Survival (%) vs Years