Phase II Trial of Idiotype Vaccination in Previously Treated Patients With Indolent Non-Hodgkin’s Lymphoma Resulting in Durable Clinical Responses


ABSTRACT

Purpose
To evaluate idiotype (Id) vaccination as a single agent in previously treated patients with indolent non-Hodgkin’s lymphoma.

Patients and Methods
Patients underwent biopsy for determination of their lymphoma-specific Id sequence. Recombinant Id protein was manufactured and covalently linked with keyhole limpet hemocyanin (KLH) to generate Id/KLH. Patients received Id/KLH 1 mg on day 1 subcutaneously, with granulocyte-macrophage colony-stimulating factor 250 μg on days 1 to 4, monthly for 6 months. Booster injections were administered until progression. Both clinical and immune responses were evaluated.

Results
Thirty-two previously treated patients received at least one injection of Id/KLH, and 31 were assessed for efficacy. Responses were observed in four patients (one complete response and three partial responses). Median time to onset of response was 5.9 months (range, 2.3 to 14.1 months). Median duration of response has not been reached but should be at least 19.4 months (range, 10.4 to 27.2+ months). Median time to progression is 13.5 months. The most common adverse events were mild to moderate injection site reactions. Six (67%) of nine patients tested demonstrated a cellular immune response, and four (20%) of 20 patients demonstrated an antibody response against their Id.

Conclusion
This trial demonstrates that Id/KLH alone can induce tumor regression and durable objective responses. Further study of Id/KLH is recommended in other settings where efficacy may be further enhanced as in first-line therapy or after cytoreductive therapy.

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INTRODUCTION

Indolent B-cell non-Hodgkin’s lymphoma (NHL) is amenable to immune intervention as evidenced by spontaneous tumor regression1 and responsiveness to immunotherapeutic strategies.2-4 Passive immunotherapy (eg, rituximab) represents an important advance for B-cell lymphomas; however, the majority of patients with indolent NHL still ultimately relapse. The induction of an active immune response represents a promising approach to the treatment of B-cell lymphomas that may result in more durable remissions.

Idiotype (Id)/keyhole limpet hemocyanin (KLH), a form of active immunotherapy, is being investigated for patients with B-cell lymphomas. Id/KLH is designed to induce an immune response to the unique Id protein found in each B-cell lymphoma.5 Prior methods used to produce patient-specific Id vaccines were difficult and time consuming.6-8 Recombinant technologies now allow for a more rapid and reliable production of Id vaccines.9,10 After successful Id vaccination, an immune response generated against a lymphoma-specific Id protein should be limited to malignant B cells, while sparing normal B cells.

Early studies have demonstrated that patients with B-cell lymphoma can mount an Id-specific immune response after Id immunization.9 Development of an anti-Id immune response correlated with an improvement in disease-free and overall survival.7
Incorporation of granulocyte-macrophage colony-stimulating factor (GM-CSF) into the Id/KLH vaccination regimen increases the frequency of detectable Id-specific cellular immunity when compared with prior studies. Most clinical trials with Id/KLH now incorporate GM-CSF into the vaccination regimen.

All prior clinical trials with Id/KLH have been conducted in patients who were first experienced remission with chemotherapy. A small number of these patients had residual disease after chemotherapy, which improved further after vaccination. The present phase II trial evaluates the antitumor activity of Id/KLH as a single agent in previously treated patients with indolent B-cell NHL who have measurable disease. The dose and schedule of Id/KLH (FavId; Favirille Inc, San Diego, CA) and GM-CSF (Leukine, Sargramostim; Berlex Laboratories, Montville, NJ) were derived from prior clinical studies using Id vaccines.

PATIENTS AND METHODS

Eligibility

Patients were accrued to this multicenter phase II trial between March 2000 and September 2002. Eligible patients had histologically confirmed indolent (low-grade or follicular) B-cell NHL of grades A through D, as defined by the International Working Group Classification, and were previously treated. Patients were required to have lymphoma accessible for biopsy, be at least 18 years old, had an Eastern Cooperative Oncology Group performance status of ≤ 2, and had adequate hematologic, renal, and hepatic function. Patients were ineligible if they had more than six (later revised to > three) prior treatment regimens, concurrent immunosuppressive therapy, or a prior splenectomy. Patients were also ineligible if they were pregnant or breast feeding, had a history of CNS lymphoma, HIV infection, or serious coexistent active medical problems, or had a history of a prior malignancy (excluding nonmelanoma carcinomas of the skin and in situ cervical carcinomas) unless the patient was in remission for ≥ 2 years. Institutional review board approval and written informed consent was obtained for all patients. The trial was registered with a public trial registry (www.ClinicalTrials.gov).

Initial Staging Evaluation and Biopsy

Pretreatment staging included a history and physical examination, CBC, blood chemistry profile, urinalysis, rheumatoid factor (RF), and computed tomography (CT) and/or magnetic resonance imaging scans of the neck, chest, abdomen, and pelvis. Bone marrow evaluation was required to confirm complete response (CR). After consent, all patients underwent a biopsy of their lymphoma for manufacture of their custom Id/KLH vaccine.

Id/KLH Production

An immunoglobulin heavy- and light-chain gene library was constructed from fresh biopsy tissue for each patient using two different constant heavy- and light-chain cDNA primers with a commercially available 5’ RACE kit (Invitrogen Corp, Carlsbad, CA). The choice of κ versus λ light-chain primers was dictated by tumor light-chain expression as determined by immunohistochemistry or flow cytometry. Variable region gene sequences that appeared multiple times from different cDNA reactions were considered tumor derived. Tumor-derived variable heavy- and light-chain sequences were cloned into a baculovirus plasmid vector containing the immunoglobulin (Ig) G1 and κ or λ constant region coding sequences. This transfer vector was introduced into Sf9 insect cells using standard methods to obtain a high titer baculovirus stock, which was then added to the Trichoplusia ni cell line for IgG1 production. The secreted full-length IgG1 antibody was purified from culture supernatant by protein A affinity chromatography followed by ion exchange chromatography. Purified antibody was conjugated to KLH at a 1:1 (weight-to-weight) ratio and formulated for subcutaneous injection as described. Using these methods, at least six doses of Id/KLH were produced for all patients treated in this trial.

Treatment and Follow-Up

The treatment schedule and follow-up evaluation is diagrammed in Figure 1. Patients received Id/KLH 1 mg (1 mL) by subcutaneous injection on day 1, along with GM-CSF 250 μg. Both Id/KLH and GM-CSF were generally split between two injection sites on day 1. On days 2 to 4, GM-CSF 250 μg was administered as close as possible to the day 1 injection site(s). This treatment was administered monthly for 6 months. Patients who had not experienced progression after six injections of Id/KLH could continue with booster injections of Id/KLH and GM-CSF every 2 months for 1 year and then every 3 months until disease progression.

Assessment of Safety and Efficacy

Safety was assessed with a monthly history and physical examination, CBC with differential, platelet count, and serum chemistries. Serum RF was monitored every 3 months. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria. Patients were observed for adverse events after each Id/KLH injection.

Efficacy was assessed every 3 months with a physical examination and CT or magnetic resonance imaging scan using the International Workshop Response Criteria. All radiographic scans were read by an independent central reviewer (W.D.C.). Responses were determined by a central committee using International Workshop Response Criteria.

Immune Response Determination

Assessment for humoral and cellular immune responses against KLH and autologous Id protein were performed on serum and peripheral-blood mononuclear cell (PBMC) preparations isolated at baseline and before each Id/KLH and GM-CSF injection.

Humoral immune response. Patient or control antibody (IgG1) was coated onto microtiter plates. Serum samples were added at sequential four-fold dilutions. Anti-Id antibodies were detected by enzyme-linked immunosorbent assay using a horseradish peroxidase–conjugated polyclonal goat anti-Cκ or anti-CA antibody, whichever was opposite to the light-chain isotype expressed by the patient’s Id antibody preparation. A response was considered positive if a four-fold increase in titer was measured against the patient’s antibody preparation compared with pretreatment levels with no cross-reactivity against an unrelated patient’s IgG1 preparation. Antibody responses to KLH were determined by adding serum samples to wells previously coated with KLH starting at a dilution of 1:64 followed by further four-fold dilutions. Anti-KLH antibodies were detected by the addition of horseradish peroxidase–conjugated polyclonal goat antihuman IgG antibody. A response was considered positive if a 16-fold increase in titer was measured compared with pretreatment levels.

Cellular immune response. PBMCs isolated from fresh heparinized blood using Ficoll Hypaque density gradient separation were flash frozen until use. PBMCs were cultured at 1 × 10^6 cells/mL in 96-well plates in the presence of Id/KLH for 5 days. Supernatants were harvested on day 5 and analyzed for the presence of interferon-γ using an enzyme-linked immunosorbent assay.
of culture media, KLH (100 μg/mL), patient derived IgG1, or control patient IgG1 (100 μg/mL). After 8 to 12 hours, Brefeldin A (2 μg/mL; BioLegend, San Diego, CA) was added, and the cells were then maintained in culture for a total of 24 hours. After culture, cells were harvested and stained with a monoclonal allophycocyanin conjugate anti-CD3 and tricolor conjugated anti-CD4. The stained cells were then fixed in 4% paraformaldehyde and stained with phycoerythrin-conjugated anti–interferon gamma, anti–tumor necrosis factor alpha, or an irrelevant IgG control. The percentage of CD3+CD4+ cells containing cytoplasmic interferon gamma or tumor necrosis factor alpha was enumerated by flow cytometry. A response was considered positive if a minimum two-fold increase in the number of cytoplasmic cytokine-expressing cells was observed over pre-Id/KLH levels and, in the case of Id-specific responses, without cross reactivity to stimulation with control IgG.

Statistical Methods

The intent-to-treat population consisted of any patient who received at least one injection of Id/KLH. Efficacy assessment was performed for all patients who had both a baseline and follow-up assessment and received at least one injection of Id/KLH.

The primary end point was overall response rate. The 95% CI for overall response rate was calculated based on the exact method of the binomial distribution. Secondary end points included time to progression (TTP) and duration of response. TTP was measured from the date of the first Id/KLH injection to the date of disease progression. Duration of response was measured from the date of the first observation of response to the date of disease progression. Median TTP was estimated using the Kaplan-Meier method. Analyses were performed with SAS statistical software, Version 8.2 (SAS Institute, Cary, NC).

RESULTS

Patient Population

Sixty-six patients were registered and evaluated for study eligibility. Sixty-two patient biopsies were received and screened for Id/KLH production. Twenty-two patients were ineligible for the following reasons: incorrect histology (n = 9), alternate treatment administered (n = 6), absence of tumor cells in the biopsy specimen (n = 5), and lack of measurable disease (n = 2). Forty patients met the eligibility criteria, and a patient-specific Id/KLH vaccine was manufactured for all 40 patients. Eight of these 40 patients never received Id/KLH on this trial because of disease progression (n = 8) or death (n = 2) before availability of Id/KLH. The characteristics of the 32 patients who received at least one dose of Id/KLH are listed in Table 1. One patient, on central radiology review, was without evidence of disease at baseline (after prior treatment with rituximab) and was not included in the efficacy evaluation. Thirty-one patients were assessed for response based on a central radiology and/or clinical evaluation. Twenty-seven patients completed both a baseline and follow-up CT scan and were assessable for changes in tumor burden over time.

Efficacy

Objective responses were identified in four (12.9%) of 31 patients (95% CI, 3.6% to 29.8%), with one CR and three PRs (Table 2). All four responders had a follicular histology. At study entry, three patients had progressive disease, and one had a stable PR after chemotherapy. Baseline tumor burden for the four responders, as measured by the sum of the products of the greatest diameters, ranged from 16 to 90 cm².

The patient who achieved a CR was a 49-year-old man with stage II disease who had a stable PR 7 months after completing cyclophosphamide, vincristine, and prednisone (CVP). After initiation of Id/KLH vaccine, a gradual reduction in overall tumor mass was observed, with attainment of a CR after six injections. Presently, this patient remains in CR at 44+ months and continues booster injections of Id/KLH every 3 months.

Of the three patients who achieved a PR, one was a 61-year-old man with a history of coronary artery disease who had experienced...
relapse after CVP chemotherapy. He demonstrated a PR at month 3 and remained in PR at the time of his death at month 25 from a myocardial infarction. The second patient was a 46-year-old man who had experienced relapse after a 20-month response to a fludarabine/tositumomab + iodine-131 tositumomab combination. After initiation of Id/KLH, the patient experienced initial disease progression at month 3, followed by a gradual reduction in tumor volume starting between months 3 and 5, with a PR achieved at month 14. According to central review, this patient had a 51% increase in the sum of the products of the greatest diameter at month 24, which is reflected in Figure 2. Central review of subsequent CT scans, after month 24, showed further tumor regression. On the basis of site assessment, the patient remains in partial remission and continues booster injections of Id/KLH at 49+ months. The third patient was a 54-year-old woman in second relapse after prior cyclophosphamide, doxorubicin, vincristine, and prednisone plus rituximab. At month 6, the patient achieved a PR, which was maintained until month 17. A repeat biopsy of her lymphoma after disease progression demonstrated disappearance of the Id clone against which her vaccine had been directed. The predominant clone in this second biopsy expressed a variable light-chain gene that differed from that expressed in the first biopsy by a single nucleotide, as determined by DNA sequencing, resulting in an amino acid substitution. The heavy chains expressed by tumor cells present in both biopsies were identical at the DNA level. This light-chain sequence had been present as a minor clonal variant in her initial biopsy.

Among the four responders, the median time to response was 5.9 months, and the median duration of response has not been reached but should be at least 19.4 months (range, 10.4 to 27.2+ months). Of the 27 patients assessed by central radiology review, with a baseline and follow-up CT scan, most of the patients had at least some reduction in their tumor measurements (Fig 3). The median TTP for the 31 patients is 13.5 months, and the median TTP for responders (CR + PR) is at least 28.8 months (range, 17.1 to 33+ months; Fig 2).

**Immunologic Responses**

Immunologic testing demonstrated a high rate of immune response to the highly immunogenic KLH. Eight (89%) of nine patients developed a T-cell anti-KLH response, whereas 20 (80%) of 25 developed a humoral anti-KLH response. Six (67%) of nine patients tested demonstrated a T-cell response, and four (20%) of 20 patients tested demonstrated a humoral anti-Id response (Table 3). Patients who became immune positive did so after a median of four Id/KLH injections (range, two to eight injections). All four patients who achieved clinical objective responses demonstrated a cellular immune response against their Id, whereas only one of these four patients developed humoral immunity (Table 4).

**Safety**

Safety was assessed in the 32 patients who received at least one Id/KLH injection (Table 5). The most common adverse event was injection site reaction (75% of patients). Injection site reactions were transient and characterized by grade 1 (mild) or grade 2 (moderate) erythema (50%), pruritus (28%), tenderness (21%), and swelling (21%). Other adverse events included headache (28%), bone pain (28%), and back pain (25%). No patient required dose reductions in either Id/KLH or GM-CSF. No cumulative toxicities were observed. One patient experienced grade 3 diarrhea while receiving oral antibiotics.

Two patients experienced lymphoma transformation. The first patient, a 61-year-old woman with relapses from both prior rituximab and prior CVP, demonstrated transformation at week 5 and died of liver failure secondary to lymphoma involvement. The second patient, a 65-year-old white man with a 10-year history of NHL who had received prior combination therapy with cyclophosphamide, doxorubicin, vincristine, and prednisone plus rituximab, developed transformation after month 6.

RF levels were monitored as a surrogate for early autoimmunity. One patient with a negative RF at baseline demonstrated an RF

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**Table 3. Immune Responses**

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<tr>
<th>Response</th>
<th>CD4 T-Cell Response (KLH, n = 9)</th>
<th>Antibody Response (KLH, n = 25)</th>
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<td>Pos. No.</td>
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| Abbreviations: KLH, keyhole limpet hemocyanin; Id, idiotype. *Patients negative for KLH were not tested for Id.
of 40 U/mL at month 7. The patient remained asymptomatic with continued Id/KLH, and subsequent RFs were negative. A second patient with an RF of 38 U/mL at baseline experienced an increase in RF to 50 U/mL at month 1 without symptoms. No subsequent RF values were obtained because the patient was removed from study with progressive disease.

**DISCUSSION**

This trial of Id/KLH as a single agent in previously treated patients with measurable indolent NHL is unique in that chemotherapy-induced clinical remission was not required before the start of vaccination.

**REFERENCES**


Hurwitz SA, Timmerman JM: Recombinant, tumour-derived idiotype vaccination for indolent B cell non-Hodgkin’s lymphomas: A focus on FavId. Expert Opin Biol Ther 5:841-852, 2005


### Authors’ Disclosures of Potential Conflicts of Interest

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO’s conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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