

# Personalized Immunotherapy for the Treatment of Non-Hodgkin Lymphoma: A Promising Approach

Julie Vose, MD

Dr. Vose is Neumann M. and Mildred E. Harris Professor and Chief, Section of Oncology/Hematology, at the University of Nebraska Medical Center in Omaha, Neb.

Address correspondence to:  
Julie Vose, MD, Neumann M. and Mildred E. Harris Professor, Chief, Section of Oncology/Hematology, University of Nebraska Medical Center, Omaha, NE 681980-7680; E-mail: [jmvose@unmc.edu](mailto:jmvose@unmc.edu).

**Abstract:** The efficacy of immunotherapeutic strategies for the treatment of lymphoid malignancies has been demonstrated in recent years. In patients with B-cell lymphomas, particularly indolent lymphoma, the use of passive immunotherapy, such as the anti-CD20 monoclonal antibody rituximab, has made an impressive impact on patient outcome. Personalized immunotherapy, a method that triggers the immune system to mount a response against tumor cells, has shown promising results in early clinical trials in hematologic malignancies. This therapeutic modality appears safe, with the most common adverse events being transient, local reactions at the site of injection. Furthermore, personalized immunotherapy has the potential to generate immunologic memory, which could provide prolonged remission. Currently, 3 large phase III studies are evaluating the efficacy and safety of personalized immunotherapy in patients with follicular lymphoma. It is hoped that the results of these studies will lead to the incorporation of this promising approach into the standard treatment of patients with lymphoma.

**N**on-Hodgkin lymphoma (NHL) represents a heterogeneous group of hematologic malignancies with diverse clinical outcomes. In 2005, it is estimated that 56,390 cases will be diagnosed, with an estimated 19,200 deaths being attributed to NHL.<sup>1</sup> Indolent lymphomas are B-cell malignancies that are associated with reasonably long survival times measured in years but are generally considered not curable by conventional therapies.<sup>2</sup> Follicular lymphoma is the most common form of indolent lymphoma and comprises 35% of adult NHL cases in the United States and 22% worldwide.<sup>3</sup> Patients most commonly present with diffuse, painless, persistent lymphadenopathy, and extranodal disease other than bone marrow involvement is uncommon. Limited-stage disease (stages I and II) represents 20% of all follicular lymphoma<sup>4</sup>; however, the majority of patients present with advanced-stage disease.<sup>5</sup>

## Keywords

Lymphoma, immunotherapy, vaccine, idiotype.

## Treatment Options for Follicular NHL

Radiation therapy is the standard therapy for patients with early-stage (stage I/II) follicular lymphoma, and it has been shown to result in 10-year overall survival rates of 64–80%.<sup>6,7</sup> Among patients with advanced asymptomatic disease, a watch-and-wait strategy is often employed. In studies comparing immediate therapy with therapy delayed until the onset of progressive (symptomatic) disease, no difference in survival was observed.<sup>8</sup> Once onset of symptoms occurs, chemotherapy, either as a single-agent (chlorambucil, cyclophosphamide, cladribine, or fludarabine) or in combination (cyclophosphamide, vincristine, and prednisone [CVP] or cyclophosphamide, doxorubicin, vincristine, and prednisone [CHOP]) has been standard.<sup>3</sup> Generally, indolent lymphomas are very sensitive to chemotherapy with complete response (CR) rates ranging from 37% to 81%.<sup>9–14</sup> However, relapse is imminent, and disease- and relapse-free periods have been shown to last for approximately 15 months to 3.6 years,<sup>13,15,16</sup> with decreasing response durations occurring with each subsequent therapy. The use of hematopoietic stem-cell transplantation has been studied in patients with relapsed follicular lymphoma; however, its role currently remains unclear.<sup>3</sup>

Agents have been developed that utilize cell surface markers expressed at high levels on B cells. The most widely used of these markers in NHL is the monoclonal anti-CD20 antibody rituximab (Rituxan, Genentech), which has been shown to produce an overall response rate of approximately 50% as a single agent in patients with relapsed or refractory disease, with a median response duration of approximately 1 year.<sup>20</sup> The current standard of care for follicular lymphoma is a combination of chemotherapy and rituximab (CHOP-R, CVP-R), which provides overall response rates of 81–97%, with response durations of approximately 32–36 months.<sup>15,18</sup> Major adverse events associated with rituximab therapy include infusion reactions (fever, rigors, and hypotension). Immunosuppression remains a concern as CD20 is expressed on normal B lymphocytes as well as on malignant cells.<sup>19,20</sup> Radioimmunoconjugates, such as ibritumomab (Zevalin, Biogen Idec) and tositumomab (Bexxar, GlaxoSmith-Kline), deliver targeted radiotherapy to B cells and have produced impressive activity in follicular NHL.<sup>21,22</sup> While standard therapy for follicular lymphoma appears to have progressed from the use of less-specific cytotoxic therapies alone, which result in the death of normal cells and systemic toxicity, to the use of more targeted therapeutic monoclonal antibodies (mAbs), more tumor-specific, less toxic alternatives are still needed. Patients with follicular lymphoma may live with their disease for years without requiring treatment. This progression-free period has led

to extensive research on the identification of prognostic features to aid clinicians in deciding which patients would best benefit from early treatment. However, the fear of over-treatment persists, highlighting the need for therapeutic options with favorable toxicity profiles. In older patients, the use of therapies with the potential for serious adverse events may impart unnecessary risk. Finally, follicular lymphoma responds well to many of the current therapeutic options, but consistently relapses and is characterized by a constant cycle of remission and relapse.<sup>2</sup> Therapeutic options with improved efficacy are needed to break this cycle and potentially provide long-term remissions.

Personalized immunotherapy, also referred to as idiotype (Id) vaccine therapy, is a patient- and tumor-specific approach. This modality targets unique protein determinants of the immunoglobulin molecules produced by the malignant B-cell clone and has been tested in phase II and phase III trials.

## Immunotherapy for the Treatment of Follicular NHL

Immunotherapy refers to the use of the immune system or its components to target and eradicate the tumor. B-cell lymphomas are considered to be the most immuneresponsive of all human cancers.<sup>21</sup> Evidence supporting this statement includes the observation that B-cell lymphomas can undergo spontaneous regression.<sup>22</sup> In addition, both complete and partial responses have been elicited through the use of nonspecific immune activators, such as bacillus Calmette-Guerin and interleukin-2.<sup>23,24</sup> Based on these data, the follicular B-cell lymphomas represent excellent candidates for immunotherapy.

The most common form of immunotherapy employed in the treatment of cancer is passive immunotherapy, which involves the administration of manufactured antibodies that target a particular antigen. Therapeutic monoclonal antibodies, such as rituximab and alemtuzumab (Campath, Berlex), are examples of a passive approach. Currently, several B-lymphocyte antigens, including CD20, CD22, and CD52, have been utilized as targets for immunotherapy. These targets, while found on lymphoma cells, are also expressed on normal immune system components, such as normal B lymphocytes.<sup>25–27</sup> Thus, the therapeutic agents that target these markers, rituximab, epratuzumab (Amgen), and alemtuzumab, while demonstrating impressive response rates, have the potential to interfere with normal immune system function.<sup>19,28,29</sup> Furthermore, while these agents are active in the treatment of follicular B-cell lymphomas, they are not curative, and patients will eventually require additional treatment.

In contrast, active immunotherapy that utilizes the Id as a target for lymphoma (personalized immunotherapy) stimulates the host's own immune system to mount a response against the tumor cells. This approach has been shown to result in improved response durations, indicating that long-term memory and potentially durable remissions with minimal toxicity may be possible.<sup>30,31</sup> Personalized immunotherapy in patients with follicular lymphoma is currently an area of intense interest.

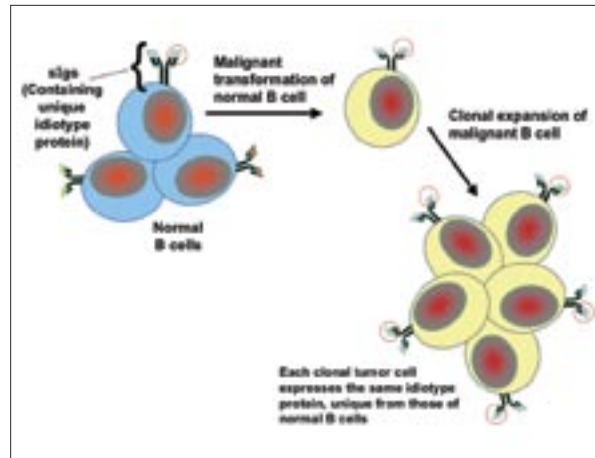
### Targeting Idiotype with Immunotherapy

Each B lymphocyte expresses an immunoglobulin molecule on its surface that is capable of recognizing and binding to a unique antigen. The region of the immunoglobulin that binds to the antigen is the product of a unique combination of gene sequences and is referred to as its Id variable region. The entire immunoglobulin and its unique variable region are commonly referred to as the Id protein (Figure 1).<sup>32</sup> B-cell malignancies are clonal in nature, and arise from a single parent B lymphocyte. When a B lymphocyte undergoes malignant transformation, the Id sequences are maintained by the malignant clones and can thus serve as a tumor-specific antigen. The Id can be recognized by antibodies as well as by receptors on T lymphocytes, making this marker an ideal target for immunotherapy.

Early studies conducted in the 1980s attempted to utilize patient-specific Id to generate murine mAbs for passive immunotherapy.<sup>33</sup> Patient tumor and myeloma cells were fused to generate stable cell lines capable of secreting large quantities of tumor-specific immunoglobulin, which contained the Id sequence. This protein was then injected into mice to produce murine anti-Id mAbs that were isolated and used to treat refractory lymphoma patients. These small early studies demonstrated promising responses. Over a 12-year period, a total of 45 patients were treated, with objective tumor regression observed in 66% of cases and 1 patient maintaining a CR for 42 months.<sup>21,33</sup> However, this production method was cumbersome and expensive. In addition, further research demonstrated that mutations within the Id sequence of the tumor cells was possible, rendering the mAbs ineffective over time.<sup>34</sup> Based on these findings, it was reasoned that an active immunotherapy approach might be more effective. By utilizing the patient's own immune system, immune responses could be generated against multiple epitopes within the Id region, as opposed to 1 epitope, as was the case with murine therapeutic mAbs.

### Personalized Immunotherapy

In early animal studies, Id isolated from murine plasmacytomas was used to immunize syngeneic mice.<sup>35</sup>



**Figure 1.** Idiotype as a tumor antigen specific for B lymphoma cells.

These studies demonstrated the ability of this method to generate an immune response mounted against the Id. Furthermore, subsequent injection of tumor cells resulted in rapid elimination of the tumor. In order to elicit an adequately strong immune response, an immunogenic carrier protein was needed.<sup>36,37</sup> Keyhole-limpet hemocyanin (KLH), a protein derived from a California giant sea snail, allowed for improved immunogenicity when chemically linked to the Id protein. KLH has since been incorporated as a standard component of most personalized immunotherapy regimens.

The first human studies of personalized immunotherapy were pioneered by Dr. Ronald Levy at Stanford University Medical Center and were conducted using the hybridoma method of Id protein production.<sup>38</sup> Patient tumor cells obtained on a lymph node biopsy were fused with a myeloma cell line. The resulting hybridoma could produce large quantities of patient-specific Id protein for treatment by subcutaneous injection. Patients enrolled in the initial trial included only those with minimal residual disease or a CR following standard chemotherapy; no chemotherapy was allowed for 6 months prior to entering the study in order to preserve patient immunocompetence and allow an anti-Id immune response. Among 9 patients treated with Id protein conjugated to KLH (Id-KLH) by subcutaneous injection, 7 mounted Id-specific immune responses. Two patients had a measurable tumor at initiation of therapy and achieved complete tumor regression. Toxicity was minimal and consisted of only mild transient local reactions at the site of injection.

Based on these promising results, enrollment in this study was extended to include a total of 41 patients with B-cell NHL.<sup>30</sup> Forty-nine percent of patients generated Id-specific immune responses. At a median follow-up of 7.3 years from diagnosis and 5.3 years from the last che-

motherapy regimen prior to personalized immunotherapy, the median duration of freedom from disease progression (FFP) for all patients was 4.4 years. Among patients who developed an antitumor Id response, median duration of FFP was 7.9 years, which was significantly greater than that observed among patients who did not mount an immune response (ie, 1.3 years;  $P < .0001$ ).

Efforts to improve the potency of personalized immunotherapy have focused on 2 approaches. Dendritic cells play a key role in the initiation of an immune response.<sup>39</sup> These cells can be isolated from peripheral blood or generated in vitro and, when combined with tumor-derived Id protein, can promote antitumor immunity. In a study that enrolled a total of 35 patients with follicular NHL, 4 of the 10 initial patients with measurable relapsed or residual disease following chemotherapy showed evidence of antitumor activity, including a CR in 2 patients.<sup>40</sup> Subsequently, 25 additional patients with advanced-stage disease were treated following chemotherapy. Among the 18 patients with residual tumor at the time of treatment with Id-pulsed dendritic cells, 22% experienced tumor regression and 16 of 23 remained without tumor progression for a median of 43 months following chemotherapy. Although this approach has been important in the development of personalized immunotherapy, it is still in the early stages of development; further research will be required to determine whether it can be utilized in a large-scale setting.

A second approach has been the use of granulocyte-macrophage colony-stimulating factor (GM-CSF) as an adjuvant to boost the magnitude of anti-Id immunity. GM-CSF is an important factor in the growth, maturation, and antigen-presenting properties of dendritic cells.<sup>39</sup> In a murine model, GM-CSF significantly enhanced the protective antitumor immunity induced by the injection of Id-KLH.<sup>41</sup>

A single-arm, phase II study of Id-KLH combined with GM-CSF was conducted by the National Cancer Institute in 20 patients with follicular lymphoma who had achieved a CR following chemotherapy.<sup>42</sup> Tumor-specific cytotoxic CD8+ and CD4+ T cells were identified in 19 of the 20 patients immunized. Among 11 patients who had detectable translocations in both primary tumor and peripheral blood following chemotherapy despite achieving a CR, 8 patients achieved a complete molecular remission following immunization. With a median follow-up of 7.2 years, 50% of the 20 patients remained in a continuous first CR, with an overall survival of 90%.<sup>31</sup> The efficacy demonstrated in this study led to the incorporation of GM-CSF into personalized immunotherapy regimens.

## Current Clinical Status of Personalized Immunotherapy

The hybridoma method used to produce therapeutic Id protein in the early studies of Id-based personalized immunotherapy was both time- and labor-intensive. In addition, this method required substantial amounts of fresh tumor and carried a significant failure rate of 15–20%.<sup>21</sup> Advances in the use of the polymerase chain reaction to amplify and clone Id genes directly from small amounts of B-cell tumor samples have resulted in new and improved methods of production of therapeutic Id protein.<sup>43,44</sup> Once cloned, Id genetic sequences can be inserted into vectors for in vitro expression of the recombinant Id (rId) protein. This recombinant technology has several advantages over the hybridoma method: it is more efficient, requires less tumor tissue, does not require viable cells for therapy development (frozen tissues can be used), and has a success rate of greater than 95%.<sup>21</sup> In addition, this technology is scalable and reproducible, making it possible to produce personalized immunotherapies for large numbers of patients.

Several clinical trials investigating the efficacy of this approach are ongoing (Table 1). In a phase II trial conducted in 22 follicular lymphoma patients in first clinical remission, rId-KLH (MyVax Personalized Immunotherapy, Genitope) plus GM-CSF produced immune responses in 13 of 21 evaluable patients (62%).<sup>45</sup> Immune responses were observed in patients regardless of whether they had achieved a complete remission (9/13) or a partial remission (4/8) on chemotherapy. Final results of this trial are expected in 2005.

In a similar phase II study conducted in 16 patients with asymptomatic, low-grade follicular lymphoma, rId-KLH combined with GM-CSF was administered to previously untreated patients during the watch-and-wait period.<sup>46</sup> Patients were treated with 5 doses of rId-KLH and GM-CSF, both for 4–6 months following tumor biopsy to allow time for the production of rId-KLH. In a preliminary report, 11 of 13 patients who developed immune response demonstrated Id-specific immune responses (9 humoral and 2 cellular responses). Of note, 4 of the 6 patients who received all 5 doses of rId-KLH at the time of this presentation experienced a mixed response or stable disease and went on to receive 3 additional immunizations. Final results of this trial are expected in 2005.

In a phase II study of rId-KLH (FavId, Faville), 88 patients with follicular lymphoma previously treated with rituximab were treated with 6 doses of rId-KLH

**Table 1.** Ongoing Personalized Immunotherapy Trials

Study	Immunotherapy	Disease State	Status
Genitope phase III	rId-KLH + GM-CSF	Follicular lymphoma in first remission following CVP	Accrual complete (Outcome of first interim analysis is expected in 2005)
NCI phase III	Id-KLH vs KLH + GM-CSF	Follicular lymphoma in first remission following PACE (CR only)	Open for accrual
Favrille phase III	rId-KLH + GM-CSF following rituximab	Treatment-naïve or refractory follicular lymphoma ( $\leq 2$ prior regimens)	Open for accrual
Favrille phase II <sup>47</sup>	rId-KLH + GM-CSF	Follicular lymphoma previously treated with rituximab	Accrual complete
UCSD phase II	rId-KLH + GM-CSF	Indolent or mantle cell lymphoma following HDCT + HSCT	Open for accrual
University of Nebraska	rId-KLH + GM-CSF	Follicular lymphoma after HDCT + HSCT	Open for accrual
Genitope phase II (2002-09) <sup>48</sup>	Rituximab followed by rId-KLH + GM-CSF	Refractory or progressive follicular lymphoma	Accruing patients from phase III study who progressed following CVP therapy*
Genitope phase II (9902) <sup>49</sup>	rId-KLH + GM-CSF	Aggressive lymphoma in first remission after CHOP or CNOP	Accrual complete (Results expected 2006)
Genitope phase II (2000-04) <sup>46</sup>	rId-KLH + GM-CSF	Low-grade lymphoma during watch and wait	Accrual complete (Results expected 2006)
Genitope phase II (9901) <sup>45</sup>	rId-KLH + GM-CSF	Previously untreated, low-grade follicular lymphoma	Accrual complete (Results expected 2006)
Genitope phase II (2000-07)	rId-KLH + abbreviated GM-CSF (500 mg, day 1, each immunization series)	Follicular lymphoma in first clinical remission	Accrual complete (Results expected 2006)

\* Currently only accruing patients from the Genitope phase III trial who did not maintain a PR following completion of CVP therapy prior to randomization to Id-KLH or KLH therapy.

CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone; CNOP = cyclophosphamide, mitoxantrone, vincristine, and prednisone; CR = complete response; CVP = cyclophosphamide, vincristine, and prednisone; GM-CSF = granulocyte-macrophage colony-stimulating factor; HDCT = high-dose chemotherapy; HSCT = hematopoietic stem cell transplantation; Id = idiotype; KLH = keyhole-limpet hemocyanin; NCI = National Cancer Institute; PACE = cisplatin, doxorubicin, cyclophosphamide, and etoposide; PR = partial response; rId = recombinant idiotype; UCSD = University of California, San Diego.

plus GM-CSF.<sup>47</sup> In a preliminary report of this trial, 72% of patients with relapsed/refractory disease had not progressed at a median follow up of 12 months. Among the 43 treatment-naïve patients, 82% had not progressed at a median follow-up of 9 months.

An ongoing phase II study is evaluating the efficacy of rId-KLH plus GM-CSF in patients with previously treated follicular lymphoma.<sup>48</sup> Enrollment is restricted to patients enrolled in the phase III trial of rId-KLH plus GM-CSF who failed initial cytoreductive chemotherapy with CVP or progressed following cytoreductive therapy prior to rId-KLH administration. Patients are treated with 4 doses of rituximab 375 mg/m<sup>2</sup> followed by a rest

period, then a series of 8 immunizations with rId-KLH given every 2 weeks.

Personalized immunotherapy is also being tested in hematologic malignancies other than follicular NHL. A phase II trial was designed to assess the immunogenicity of rId-KLH immunotherapy in patients with aggressive NHL, including follicular large cell, diffuse large B-cell, and mantle cell lymphomas. In a preliminary report by Leonard and colleagues, 27 patients were enrolled.<sup>49</sup> Because patients with aggressive B-cell NHL tend to relapse sooner following therapy compared with patients with indolent disease, Id-KLH therapy was initiated 3 months following CHOP chemotherapy, rather than

after the typical 6-month rest period commonly used in follicular lymphomas. Two treatment regimens were used: Schedule A consisted of 5 rId-KLH treatments over a 24-week period (N=14), while Schedule B consisted of 8 rId-KLH treatments over an 18-week period (N=13). For patients treated with Schedule A, a median time to disease progression of 10.8 months was observed, which suggests that this schedule did not allow for a clinically effective anti-Id immune response to develop rapidly enough to compete with the regrowth of the aggressive tumor.<sup>50</sup> In contrast, Schedule B provided a median time to disease progression of 15.7 months. Final results of this trial are expected soon.

The currently reported phase II studies have demonstrated that recombinant methods of Id production can produce immune response rates comparable to those achieved using the older hybridoma method (Table 2).<sup>30,40,42,45-47,49,51</sup> Also of note, personalized immunotherapy has been shown to produce anti-Id immune responses in patients with a variety of tumor types, including follicular, diffuse large B-cell, and mantle cell lymphomas,<sup>49</sup> as well as in a variety of settings, including during the watch-and-wait period,<sup>40</sup> following cytoreductive chemotherapy<sup>45</sup> or treatment with passive immunotherapy such as rituximab.<sup>47</sup> Thus, in phase II studies personalized immunotherapy has been shown to be safe and capable of generating tumor-specific immune responses, leading to the development of several phase III trials designed to confirm this finding.

### Phase III Trials of Personalized Immunotherapy

Currently, 3 phase III trials are ongoing to confirm the efficacy of personalized anti-Id active immunotherapy in patients with NHL. In general, all 3 studies are following similar designs whereby, following diagnosis, patients undergo tumor biopsy for Id manufacturing and receive cytoreduction (Figure 2). Following a rest period, a series of Id-KLH injections are given followed by GM-CSF administration. Patient follow-up is then conducted at predetermined intervals. The most advanced of the phase III studies is being conducted by Genitope Corporation.<sup>52</sup> This study is a multicenter, randomized, double-blind study evaluating previously untreated patients with stage III or IV follicular NHL.<sup>52</sup> Patients who maintain at least a partial response for 6 months following 8 cycles of therapy with CVP are randomized 2:1 to receive 7 rId-KLH plus GM-CSF immunizations over a 24-week period versus KLH plus GM-CSF. For this trial, rIds are manufactured using Hi-GET (Genitope) technology, a gene expression platform, and are expressed in mouse cells. Enrollment in this trial has been completed. The outcome of the first

interim analysis is expected in 2005, with the second interim analysis expected in 2006.

A similar phase III study is being conducted by the National Cancer Institute and Biovest International (a subsidiary of Accentia Biopharmaceuticals). This trial, being carried out at 8 medical centers in the United States, has targeted enrollment of 563 patients.<sup>44</sup> Patients who achieve a CR following standard therapy with cyclophosphamide, doxorubicin, etoposide, and prednisone are randomized to Id-KLH therapy plus GM-CSF or KLH plus GM-CSF initiated 6–12 months following chemotherapy. The Ids for this study are produced using the first-generation technology rescue hybridoma method. Enrollment in this study is ongoing.

In a randomized, double-blind, controlled phase III study being conducted by Favril, Inc., patients with either treatment-naïve or relapsed/refractory follicular NHL, following no more than 2 chemotherapy regimens, are treated with 4 weekly doses of rituximab.<sup>53</sup> Patients who do not demonstrate progressive disease after 2 months of follow up are randomized 1:1 to receive GM-CSF plus either rId-KLH or KLH plus GM-CSF. Id protein for this study is produced with recombinant methods that utilize insect cells. Accrual of 342 patients is planned, with over 60 centers participating.

### Comparison of Personalized Immunotherapy to Other Therapeutic Modalities

To date, no curative therapy exists for the treatment of follicular B-cell NHL. Cytotoxic chemotherapy and passive immunotherapy are initially effective, but patients ultimately relapse and require retreatment. It is hoped that through the development of a host-generated, specific antitumor immune response, personalized immunotherapy may produce long-term remissions and possibly even a cure. Another advantage with personalized immunotherapy compared to other therapeutic options for the treatment of follicular lymphoma is the low toxicity observed with personalized immunotherapy. In several phase II studies, the most common adverse events reported were local skin reactions at the injection site.<sup>54-56</sup> In contrast, commonly used chemotherapy regimens can produce severe gastrointestinal, neurologic, and hematologic adverse events, including neutropenia, thrombocytopenia, and anemia.

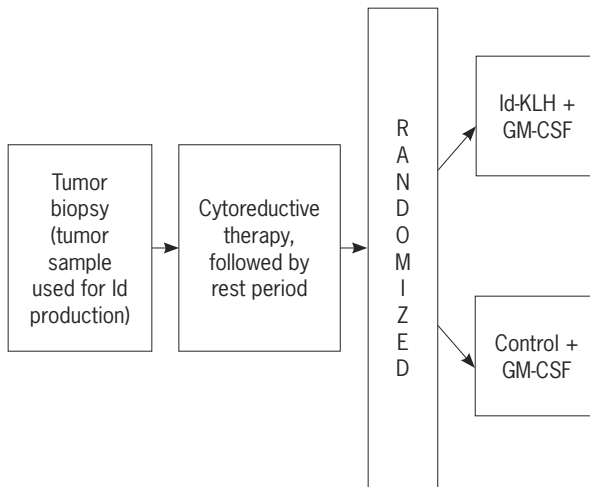
Passive immunotherapy, particularly with rituximab, has proven effective and more targeted than systemic chemotherapy, which results in reduced frequency of severe toxicities when used as monotherapy.<sup>17,19,20</sup> However, several differences in passive and active immunotherapy approaches should be noted (Table 3). Passive immunotherapy can result in direct killing of tumor

**Table 2.** Phase II Studies of Personalized Immunotherapy: Efficacy

Study	Regimen	Tumor Type	Results
Hsu et al <sup>30</sup> (N=41)	Id-KLH (hybridoma production method)	B-cell NHL	FFP median duration 4.4 years (all patients)  FFP median duration 7.9 years (among patients with an anti-Id response; $P < .0001$ )
Genitope 9901 <sup>45</sup> (N=21)	rId-KLH + GM-CSF	Follicular lymphoma	13/21 patients had Id-specific immune response (7 humoral, 2 cellular, 4 both)
Genitope 2000-04 <sup>46</sup> (N=13)	rId-KLH + GM-CSF	Low-grade follicular lymphoma during the watch-and-wait period	11/13 patients had Id-specific immune response (9 humoral, 2 cellular)
Favrille <sup>47</sup> (N=88)	Rituximab, rest period, rId-KLH + GM-CSF	Follicular lymphoma	Relapsed/refractory (N=45): 72% had not progressed at median 12-month follow up  Treatment naive (N=43): 82% had not progressed at median 9-month follow up
Genitope 9902 <sup>49</sup> (N=27)	rId-KLH + GM-CSF	Aggressive NHL	Schedule A (Id-KLH × 5 over 24 weeks): median TTP 10.8 months  Schedule B (Id-KLH × 8 over 18 weeks): median TTP 15.7 months*
Bendandi et al <sup>42</sup> (N=20)	Id-KLH (hybridoma production method) + GM-CSF	Follicular lymphoma	8/11 patients with detectable translocations converted to PCR-negative  19/20 had tumor-specific T cells
Timmerman et al <sup>40</sup> (N=35)	DCs pulsed with Id or Id-KLH (hybridoma production method) + GM-CSF  Booster with Id-KLH (6 patients who progressed on DC vaccination)	Follicular lymphoma	Initial 10 patients: 8/10 T-cell response 2 CRs, 1 PR  Next 25 patients: 15/23 T-cell or humoral response 22% tumor regression 70% stable disease  Booster Id-KLH: 2/6 CR, 1/6 PR
Neelapu et al <sup>51</sup> (N=10)	Id + liposomal lymphokine	Follicular lymphoma	Autologous tumor response 10/10 Type I cytokine response 9/10 6/10 CR

\* Reported in 13 patients treated at alternative dose of Id-KLH initiated at 13 weeks post-chemotherapy and given every 2 weeks for 7 weeks.

CR = complete remission; DC = dendritic cell; FFP = freedom from disease progression; GM-CSF = granulocyte-macrophage colony-stimulating factor; Id = idiotype; KLH = keyhole-limpet hemocyanin; NHL = non-Hodgkin lymphoma; PCR = polymerase chain reaction; PR = partial response; rId = recombinant Id protein; TTP = time to progression.



**Figure 2.** Generalized treatment schema of ongoing phase III trials of personalized immunotherapy for the treatment of follicular lymphoma.

GM-CSF = granulocyte-macrophage colony-stimulating factor; Id = idiotype; KLH = keyhole-limpet hemocyanin.

**Table 2.** Personalized Active Immunotherapy Versus Passive Immunotherapy

Personalized Active Immunotherapy	Passive Immunotherapy
Tumor specific	Not tumor specific
Stimulates host immune response	Does not stimulate host immune response
Induces immunologic memory	Temporary antitumor effect
May produce long-term immunity	Requires retreatment
Induces both the cellular and humoral arms of the immune system	Induces the humoral arm of the immune system only (ADCC, CDC)
Requires patient tumor sample for production	Does not require patient tumor sample

ADCC = antibody-dependent cellular cytotoxicity; CDC = complement-dependent cytotoxicity.

cells, either via antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity.<sup>57</sup> However, this killing occurs via the humoral arm of the immune system only, and no cellular components are engaged. The effects of passive immunotherapeutics are temporary, and retreatment is required to maintain the antitumor effect. Currently utilized passive immunotherapeutics, including rituximab and alemtuzumab, are not fully tumor-specific and target antigens found on normal lymphocytes as well.<sup>25-27</sup> Thus, while passive immunotherapies, including rituximab and alemtuzumab, are less toxic than chemotherapy, these agents can nevertheless result in prolonged effects on the immune system through the depletion of normal lymphocytes. Other toxicities associated with passive immunotherapy include infusion reactions, such as fever, chills, and tremors, which can in some cases be severe and even life-threatening.<sup>19,29</sup>

Personalized immunotherapy, in contrast, is tumor-specific and does not appear to result in depletion of normal lymphocytes or subsequent impairment of the immune system.<sup>44</sup> This technique stimulates the patient’s immune system to attack the tumor through the use of both the humoral and cellular arms of the immune system. As a result, immunologic memory may be established, which could translate into long-term remission. A patient tumor sample is required for the production of personalized immunotherapy; however, in contrast to older methods of Id-KLH development, newer production methods allow the use of frozen tissue that can be obtained at diagnostic biopsy.

### Future Directions

The results of current clinical trials of personalized immunotherapy are eagerly awaited, with the hope that this therapeutic modality will provide improved clinical outcomes for patients with follicular lymphoma as demonstrated in earlier trials. While personalized immunotherapy has been shown to result in tumor-specific immune responses, the correlation of these responses with improved clinical outcome is needed. It is speculated that patients will obtain additional benefits and prolonged duration of response through the optimization of the immunization regimen in terms of both dosing frequency and duration. Further studies to confirm this hypothesis are needed. Efforts have been undertaken by the University of Nebraska to evaluate the optimal dose of GM-CSF in combination with rId-KLH. In a phase II study of 11 patients with follicular lymphoma in first clinical remission, 500 µg of GM-CSF was administered on day 1 of each immunization series, in contrast to the standard 250 µg of GM-CSF given on days 1–4.<sup>58</sup> Five of the 11 evaluable patients (45%) demonstrated specific

anti-Id humoral immune responses. Further investigations to optimize the dosing of GM-CSF will be needed. In addition, studies to determine where personalized immunotherapy best fits into the global therapeutic strategy for the management of follicular B-cell lymphoma are also warranted.

Even as the results of current phase III studies are awaited, new advances in tumor immunology are being applied to early-stage clinical trials in order to improve the potency of personalized immunotherapy. Id protein on B-cell NHL tumor will remain an important therapeutic target, based on its exquisite degree of specificity to tumor cells; however, a greater repertoire of target antigens is desired. The use of whole cells as immunotherapy is being investigated, including the use of tumor cells transduced to express GM-CSF<sup>59</sup> or costimulatory molecules,<sup>60</sup> as well as the use of whole dendritic cells pulsed with dead tumor cells.<sup>61</sup>

Recent investigations have suggested that immunoglobulin G Fc receptor polymorphisms can affect the affinity of these receptors for the Fc portion of antibodies. In a phase II study evaluating 136 patients with follicular lymphoma who were treated with Id immunizations at Stanford University between 1988 and 2000, polymorphisms significantly correlated with clinical outcome.<sup>54,62</sup> This study suggests that such polymorphisms may shed light on how to improve the efficacy of personalized immunotherapy in the future.

One of the obstacles faced for Id-KLH personalized immunotherapy is overcoming tumor-induced immunosuppression and tolerance.<sup>63</sup> This phenomenon has been widely observed in cancer patients where the presence of a large tumor burden suppresses the immune system, overwhelming tumor-specific immunity. The phenomenon of tumor-induced immunosuppression has led investigators to focus personalized immunotherapy efforts on patients who have been cytoreduced and generally have only minimal residual disease. The recent investigation of regulatory T (Treg) cells may provide insights to overcome this obstacle. Treg cells act to inhibit the immune response in vivo, through the suppression of T cell proliferation.<sup>64</sup> It is possible that the depletion of Treg cells may allow for a more robust immune response without the need for extensive cytoreduction typically used prior to Id.

## Summary

Personalized immunotherapy may prove to be a safe and well tolerated therapeutic option for patients with indolent B-cell lymphomas. Encouraging clinical efficacy has been seen with early trials. Unlike current passive immunotherapy, this active approach is truly tumor-specific and does not adversely affect normal bystander cells. Furthermore,

this approach may result in long-term antitumor immunity and remission by virtue of immunologic memory. In contrast, passive immunotherapy requires retreatment upon eventual relapse. It is important to note that in this patient population, personalized immunotherapy appears to be safe, with the most common adverse event being a transient local skin reaction.

Preliminary studies have shown that personalized immunotherapy can be integrated with standard therapies, and this treatment does not preclude the use of subsequent therapies, if needed. However, care should be taken with prior therapies to ensure an intact immune system and optimal anti-Id response.

The use of personalized immunotherapy for the treatment of follicular B-cell lymphoma and other B-cell malignancies holds great promise. The results of randomized, phase III studies are eagerly awaited, and it is hoped that these studies will provide confirmation of the efficacy and safety of this approach.

## References

1. Jemal A, Murray T, Ward E, et al. Cancer statistics, 2005. *CA Cancer J Clin.* 2005;55:10-30.
2. McLaughlin P. Progress and promise in the treatment of indolent lymphomas. *Oncologist.* 2002;7:217-225.
3. Ganti AK, Bociek RG, Bierman PJ, et al. Follicular lymphoma: expanding therapeutic options. *Oncology (Williston Park).* 2005;19:213-228; discussion 228, 233-236, 239.
4. Horning SJ. Natural history of and therapy for the indolent non-Hodgkin's lymphomas. *Semin Oncol.* 1993;20:75-88.
5. Anderson T, Chabner BA, Young RC, et al. Malignant lymphoma. 1. The histology and staging of 473 patients at the National Cancer Institute. *Cancer.* 1982;50:2699-2707.
6. MacManus MP, Hoppe RT. Is radiotherapy curative for stage I and II low-grade follicular lymphoma? Results of a long-term follow-up study of patients treated at Stanford University. *J Clin Oncol.* 1996;14:1282-1290.
7. Fung CY, Tarbell NJ, Lucarelli MJ, et al. Ocular adnexal lymphoma: clinical behavior of distinct World Health Organization classification subtypes. *Int J Radiat Oncol Biol Phys.* 2003;57:1382-1391.
8. Ardeshta KM, Smith P, Norton A, et al. Long-term effect of a watch and wait policy versus immediate systemic treatment for asymptomatic advanced-stage non-Hodgkin lymphoma: a randomised controlled trial. *Lancet.* 2003;362:516-522.
9. Ezdinli EZ, Anderson JR, Melvin F, et al. Moderate versus aggressive chemotherapy of nodular lymphocytic poorly differentiated lymphoma. *J Clin Oncol.* 1985;3:769-775.
10. Lister TA, Cullen MH, Beard ME, et al. Comparison of combined and single-agent chemotherapy in non-Hodgkin's lymphoma of favourable histological type. *Br Med J.* 1978;1:533-537.
11. Kimby E, Björkholm M, Gahrton G, et al. Chlorambucil/prednisone vs. CHOP in symptomatic low-grade non-Hodgkin's lymphomas: a randomized trial from the Lymphoma Group of Central Sweden. *Ann Oncol.* 1994;5(suppl 2):67-71.
12. McLaughlin P, Cabanillas F, Hagemester FB, et al. CHOP-Bleo plus interferon for stage IV low-grade lymphoma. *Ann Oncol.* 1993;4:205-211.
13. Peterson BA, Petroni GR, Frizzera G, et al. Prolonged single-agent versus combination chemotherapy in indolent follicular lymphomas: a study of the cancer and leukemia group B. *J Clin Oncol.* 2003;21:5-15.
14. Kennedy BJ, Bloomfield CD, Kiang DT, et al. Combination versus successive single agent chemotherapy in lymphocytic lymphoma. *Cancer.* 1978;41:23-28.
15. Hiddemann W, Dreyling M, Forstpointner R, et al. Combined immuno-chemotherapy (R-CHOP) significantly improves time to treatment failure in first line therapy of follicular lymphoma—results of a prospective randomized trial of the German Low Grade Lymphoma Study Group (GLSG). *Blood.* 2003;102:104a. Abstract 352.

16. Marcus R, Imrie K, Belch A, et al. An international multi-centre, randomised, open-label phase III trial comparing rituximab added to CVP chemotherapy to CVP chemotherapy alone in untreated stage III/IV follicular non-Hodgkin's lymphoma. *Blood*. 2003;102:28a. Abstract 87.
17. Winter JN, Gascoyne RD, Van Besien K. Low-grade lymphoma. *Hematology* (Am Soc Hematol Educ Program). 2004;203-220.
18. Marcus R, Imrie K, Belch A, et al. CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. *Blood*. 2005;105:1417-1423.
19. McLaughlin P, Grillo-Lopez AJ, Link BK, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J Clin Oncol*. 1998;16:2825-2833.
20. Czuczman MS, Grillo-Lopez AJ, White CA, et al. Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. *J Clin Oncol*. 1999;17:268-276.
21. Timmerman JM. Immunotherapy for lymphomas. *Int J Hematol*. 2003;77:444-455.
22. Krikorian JG, Portlock CS, Cooney P, et al. Spontaneous regression of non-Hodgkin's lymphoma: a report of nine cases. *Cancer*. 1980;46:2093-2099.
23. Biologic response modifiers: a report. *Natl Cancer Inst Monogr*. 1983;63:57-59.
24. Fefer A. Interleukin-2: clinical applications--hematologic malignancies. In: Rosenberg SA, ed. *Principles and Practice of the Biologic Therapy of Cancer*. Philadelphia, PA: Lippincott Williams & Wilkins; 2000:83-92.
25. Tedder TF, Tuscano J, Sato S, et al. CD22, a B lymphocyte-specific adhesion molecule that regulates antigen receptor signaling. *Annu Rev Immunol*. 1997;15:481-504.
26. Tedder TF, Engel P. CD20: a regulator of cell-cycle progression of B lymphocytes. *Immunol Today*. 1994;15:450-454.
27. Domagala A, Kurpisz M. CD52 antigen—a review. *Med Sci Monit*. 2001;7:325-331.
28. Coleman M, Goldenberg DM, Siegel AB, et al. Epratuzumab: targeting B-cell malignancies through CD22. *Clin Cancer Res*. 2003;9:3991S-3994S.
29. Uppenkamp M, Engert A, Diehl V, et al. Monoclonal antibody therapy with CAMPATH-1H in patients with relapsed high- and low-grade non-Hodgkin's lymphomas: a multicenter phase I/II study. *Ann Hematol*. 2002;81:26-32.
30. Hsu FJ, Caspar CB, Czerwinski D, et al. Tumor-specific idiotype vaccines in the treatment of patients with B-cell lymphoma—long-term results of a clinical trial. *Blood*. 1997;89:3129-3135.
31. Neelapu S, Gause BL, Kikcevic D, et al. Vaccine therapy of follicular lymphoma in first remission: long-term follow-up of phase II results and high rate of chemotherapy-induced complete remissions in a controlled, randomized phase III trial. *Blood*. 2003;102:307b. Abstract 4953.
32. Stevenson GT, Stevenson FK. Antibody to a molecularly-defined antigen confined to a tumour cell surface. *Nature*. 1975;254:714-716.
33. Meeker TC, Lowder J, Maloney DG, et al. A clinical trial of anti-idiotype therapy for B cell malignancy. *Blood*. 1985;65:1349-1363.
34. Meeker T, Lowder J, Cleary ML, et al. Emergence of idiotype variants during treatment of B-cell lymphoma with anti-idiotype antibodies. *N Engl J Med*. 1985;312:1658-1665.
35. Lynch RG, Graff RJ, Sirisinha S, et al. Myeloma proteins as tumor-specific transplantation antigens. *Proc Natl Acad Sci U S A*. 1972;69:1540-1544.
36. Campbell MJ, Carroll W, Kon S, et al. Idiotype vaccination against murine B cell lymphoma: humoral and cellular responses elicited by tumor-derived immunoglobulin M and its molecular subunits. *J Immunol*. 1987;139:2825-2833.
37. Kaminski MS, Kitamura K, Maloney DG, et al. Idiotype vaccination against murine B cell lymphoma: inhibition of tumor immunity by free idiotype protein. *J Immunol*. 1987;138:1289-1296.
38. Kwak LW, Campbell MJ, Czerwinski DK, et al. Induction of immune responses in patients with B-cell lymphoma against the surface-immunoglobulin idiotype expressed by their tumors. *N Engl J Med*. 1992;327:1209-1215.
39. Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. *Annu Rev Immunol*. 2000;18:767-811.
40. Timmerman JM, Czerwinski DK, Davis TA, et al. Idiotype-pulsed dendritic cell vaccination for B-cell lymphoma: clinical and immune responses in 35 patients. *Blood*. 2002;99:1517-1526.
41. Kwak LW, Young HA, Pennington RW, et al. Vaccination with syngeneic, lymphoma-derived immunoglobulin idiotype combined with granulocyte/macrophage colony-stimulating factor primes mice for a protective T-cell response. *Proc Natl Acad Sci U S A*. 1996;93:10972-10977.
42. Bendandi M, Gocke CD, Kobrin CB, et al. Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma. *Nat Med*. 1999;5:1171-1177.
43. Hawkins RE, Zhu D, Ovecka M, et al. Idiotypic vaccination against human B-cell lymphoma. Rescue of variable region gene sequences from biopsy material for assembly as single-chain Fv personal vaccines. *Blood*. 1994;83:3279-3288.
44. Timmerman JM. Therapeutic idiotype vaccines for non-Hodgkin's lymphoma. *Adv Pharmacol*. 2004;51:271-293.
45. Timmerman J, Levy R, Vose J, et al. A phase 2 trial to evaluate the rate of immune response using recombinant idiotype for treatment of follicular non-Hodgkin's lymphoma. *J Immunol*. 2001;24:514.
46. Timmerman J, Levy R, Czerwinski D, et al. A phase 2 trial to evaluate the efficacy of recombinant idiotype vaccines in untreated follicular non-Hodgkin's lymphoma in the "watch-and-wait" period. *Proc Am Soc Clin Oncol*. 2002;21:4a. Abstract 13.
47. Koc O, Redfern C, Wierkniek P. Id/KLH vaccine (FavId) following treatment with rituximab: an analysis of response rate improvement (RRI) and time to progression (TTP) in follicular lymphoma. *Blood*. 2004;104:170a. Abstract 587.
48. Genitope Web site. Genitope Protocol 2002-09. Available at: <http://www.genitope.com/tr2009.html>. Accessed November 18, 2005.
49. Leonard J, Vose J, Timmerman J, et al. Recombinant idiotype-KLH vaccination (MyVax) following CHOP chemotherapy in mantle cell lymphoma. *Blood*. 2003;102:105a. Abstract 357.
50. Genitope Web site. Genitope Annual Report. Available at [http://library.corporate-ir.net/library/14/142/142124/items/149418/GTOP\\_AnnualReport.pdf](http://library.corporate-ir.net/library/14/142/142124/items/149418/GTOP_AnnualReport.pdf). Accessed November 18, 2005.
51. Neelapu SS, Baskar S, Gause BL, et al. Human autologous tumor-specific T-cell responses induced by liposomal delivery of a lymphoma antigen. *Clin Cancer Res*. 2004;10:8309-8317.
52. Genitope Web site. Genitope Protocol 2000-03. Available at: <http://www.genitope.com/tr2003.html>. Accessed November 18, 2005.
53. Hurvitz SA, Timmerman JM. Recombinant, tumour-derived idiotype vaccination for indolent B cell non-Hodgkin's lymphomas: a focus on FavId. *Expert Opin Biol Ther*. 2005;5:841-852.
54. Weng WK, Czerwinski D, Timmerman J, et al. Clinical outcome of lymphoma patients after idiotype vaccination is correlated with humoral immune response and immunoglobulin G Fc receptor genotype. *J Clin Oncol*. 2004;22:4717-4724.
55. Koene HR, Kleijer M, Algra J, et al. Fc gammaRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. *Blood*. 1997;90:1109-1114.
56. Timmerman J, Vose J, Kunkel L, et al. A phase 2 study demonstrating recombinant idiotype vaccine elicits specific anti-idiotype immune responses in aggressive non-Hodgkin's lymphoma. *Blood*. 2001;100:341a.
57. Eisenbeis CF, Caligiuri MA, Byrd JC. Rituximab: converging mechanisms of action in non-Hodgkin's lymphoma? *Clin Cancer Res*. 2003;9:5810-5812.
58. Vose J, Bierman P, Hollingsworth M, et al. A phase 2 trial to evaluate the efficacy of recombinant idiotype vaccine with abbreviated course of granulocyte-macrophage colony-stimulating factor adjuvant in follicular non-Hodgkin's lymphoma. *Blood*. 2002;100:361a. Abstract 1397.
59. Borrello IM, Sotomayor EM. Cancer vaccines for hematologic malignancies. *Cancer Control*. 2002;9:138-151.
60. Briones J, Timmerman JM, Panicelli DL, et al. Antitumor immunity after vaccination with B lymphoma cells overexpressing a triad of costimulatory molecules. *J Natl Cancer Inst*. 2003;95:548-555.
61. Gatz E, Okada CY. Tumor cell lysate-pulsed dendritic cells are more effective than TCR Id protein vaccines for active immunotherapy of T cell lymphoma. *J Immunol*. 2002;169:5227-5235.
62. Weng WK, Levy R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *J Clin Oncol*. 2003;21:3940-3947.
63. Haasz R, Robinson E. Lymphocyte reactivity in healthy subjects and cancer patients. *Acta Haematol*. 1977;57:331-338.
64. O'Garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. *Nat Med*. 2004;10:801-805.