

Expert Opinion

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BiovaxID™: a personalized therapeutic cancer vaccine for non-Hodgkin's lymphoma

Seung-Tae Lee, Yun-Fang Jiang, Keon-Uk Park, Alison F Woo & Sattva S Neelapu[†]

[†]The University of Texas M. D. Anderson Cancer Center, Department of Lymphoma and Myeloma, Division of Cancer Medicine, 1515 Holcombe Blvd, Unit 903, Houston, TX, 77030 USA

The clonal immunoglobulin molecule, idiotype (ID), expressed on the surface of B-cell malignancies can function as a tumor-specific antigen. BiovaxID™ is a patient-specific therapeutic cancer vaccine composed of the tumor idiotype conjugated to a carrier protein, keyhole limpet hemocyanin (KLH). In a Phase II clinical trial, administration of ID-KLH vaccine together with granulocyte-macrophage colony-stimulating factor to follicular lymphoma patients in complete remission induced tumor-specific cellular and humoral immunity and molecular remissions, and was associated with prolonged disease-free survival. A randomized, double-blind, Phase III clinical trial is ongoing to definitively determine the clinical benefit of BiovaxID plus granulocyte-macrophage colony-stimulating factor vaccination in patients with follicular lymphoma.

Keywords: BiovaxID™, clinical trials, idiotype, immunotherapy, lymphoma, rituximab, vaccine

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1. Introduction

Non-Hodgkin's lymphoma (NHL) is the seventh most commonly diagnosed cancer and the sixth leading cause of cancer death in the US [1]. The incidence of NHL has markedly increased over the last four decades. In 2006, an estimated 58,870 patients will be diagnosed, and an estimated 18,840 will die because of NHL in the US [1]. The clinical course of NHL can be either aggressive or indolent, depending on the subtype of lymphoma. Diffuse large B-cell lymphoma is the most common aggressive NHL, comprising ~ 30% of all NHL cases worldwide. Although diffuse large B-cell lymphoma is curable with combination chemotherapy in early or advanced stage, a significant proportion of patients die of progressive or relapsed disease. In contrast, follicular lymphoma, the most common low-grade NHL, which comprises 22% of all NHL cases worldwide [2], is potentially cured with radiation therapy only in early stages. Approximately 10 – 15% of patients with follicular lymphoma present with stage I or II disease, and the remaining present with widespread disease in the lymph nodes, bone marrow and/or spleen at diagnosis. Despite the advanced stage, the clinical course is generally indolent with a median survival of 8 – 10 years. Although highly responsive to various therapies, such as chemotherapy, radiation therapy and/or biological therapy, advanced disease is characterized by repeated remissions and relapses, and is considered incurable with the available treatment options [2]. Other indolent and aggressive lymphomas, such as marginal zone B-cell lymphomas and mantle cell lymphoma, respectively, are also generally considered to be incurable in the advanced stage. The recent inclusion of rituximab, an anti-CD20 monoclonal antibody, in induction combination chemotherapy regimens has improved the response rates, duration of remission, progression-free survival and overall survival of most B-cell NHLs. However, these combinations do not appear to be curative for advanced-stage follicular or mantle

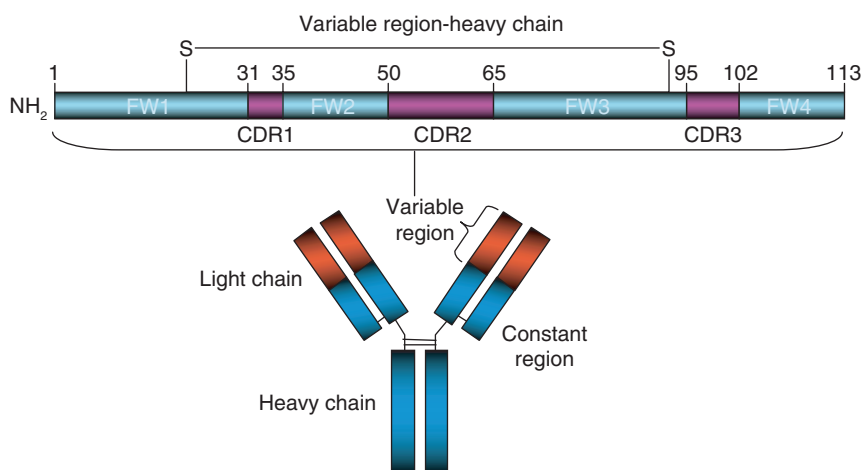


Figure 1. Idiotype as a tumor-specific antigen for B-cell lymphoma. Immunoglobulin molecules expressed on the surface of malignant B cells are composed of heavy and light chains, which possess highly specific variable regions at their amino termini and constant regions at their carboxy termini. The variable regions contain unique determinants, termed ID, that can be recognized as an antigen. The unique antigenic determinants are most likely derived from the hypervariable CDRs, but not framework regions of the variable regions of heavy and light chains [23]. Amino acid numbers in the variable heavy chain region are shown from the amino (NH₂) terminal end.

CDR: Complementarity-determining region; ID: Idiotype.

cell lymphoma, as no plateau in the survival curve has been demonstrable [3-6]; therefore, novel treatment options are needed to improve clinical outcome in these lymphomas. This review focusses on the development of an active immunotherapeutic strategy, BiovaxID™, for the treatment of B-cell NHLs.

2. Idiotype as a tumor-specific antigen for B-cell non-Hodgkin's lymphoma

The central hypothesis of active immunotherapy of cancer is that either the tumor cell itself or antigens derived from the tumor cell (which are specific, or at least selective, for the tumor cell) can be modified and injected into the patient as a therapeutic (not preventive) vaccine. The desired result is the activation of both major arms of the immune response, the host antibody response and a potential host T-cell response against the target tumor cell or antigen, thereby aiding in eradication of the disease. Several lines of evidence suggest that follicular lymphoma may be highly sensitive to immunotherapy. First, spontaneous remissions lasting > 1 year have been observed in up to 23% of patients [7]. Second, administration of the single agent rituximab monoclonal antibody to previously untreated follicular lymphoma patients has resulted in clinical responses in > 70% of patients [8,9]. Third, the survival of patients with follicular lymphoma appeared to correlate with the gene expression signatures of infiltrating non-malignant immune cells in the tumor [10]. Taken together, these data support the development of novel therapeutic vaccination strategies for the treatment of this lymphoma.

Many efforts toward developing a vaccine against human malignancies have been frustrated by the lack of identification of a tumor-specific antigen that would allow tumor cells to be distinguished from normal cells. In the case of B-cell NHL, however, the tumor-specific immunoglobulin expressed on the surface of malignant B cells can function as a tumor-specific antigen and has been exploited as a target for active immunotherapy. Each B lymphocyte expresses an immunoglobulin molecule on its surface, capable of recognizing and binding to a unique antigen. The variable 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 idiotype (ID) (Figure 1). As B-cell NHLs are composed of a clonal proliferation of mature resting and reactive lymphocytes that can express synthesized immunoglobulins on their cell surface, the idiotypic determinants of the surface immunoglobulin of a B-cell lymphoma can serve as a tumor-specific marker for the malignant clone.

3. Synthesis of idiotype vaccine for clinical use

For clinical use, the ID protein is traditionally produced by the rescue hybridoma method, described as follows (Figure 2). The lymph node biopsy sample obtained from the patient is placed in sterile cell culture media and shipped at cold temperature to the biopsy processing lab within 24 h. The tissue is minced and sieved to a single cell suspension and cryopreserved. The malignant lymphocytes from the biopsy are fused with a hypoxanthine-aminopterin-thymidine-sensitive

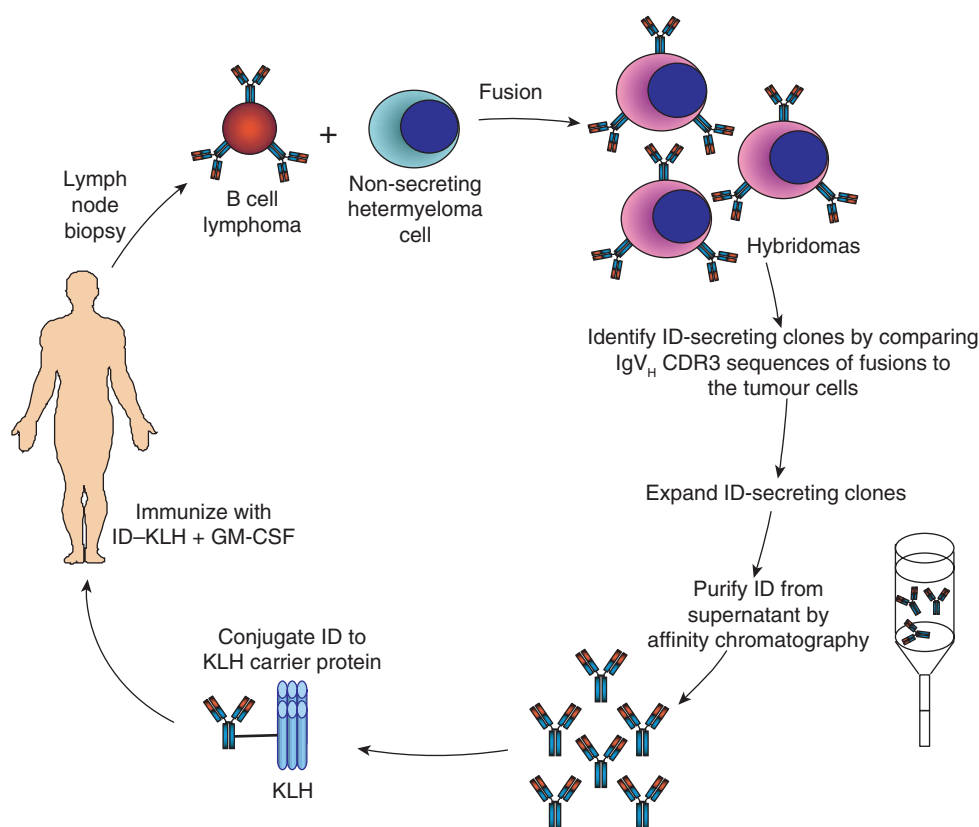


Figure 2. Schematic diagram showing the production of BiovaxID using hybridoma technology. Lymphoma tumor cells obtained from lymph node biopsy are fused to immortal heteromyeloma cells. ID-secreting hybridoma clones are identified by the IgV_H CDR3 sequence and expanded in an automated bioreactor unit. The ID is purified from culture supernatant by affinity chromatography, conjugated to the carrier protein KLH, and administered together with GM-CSF to the patient.

CDR3: Complementarity-determining region 3; GM-CSF: Granulocyte-macrophage colony-stimulating factor; ID: Idiotypic; IgV_H: Immunoglobulin heavy chain variable region; KLH: Keyhole limpet hemocyanin.

heterohybridoma K6H6/B5 to immortalize the cells and enable them to secrete the unique antibody (ID) expressed only on the surface of the malignant cells. The fused cells are cultured, and samples of cell culture supernatant are screened for the presence of secreted antibody of the same isotype as the tumor. Colonies from positive wells are fed and expanded until cell lines from individual wells can be weaned from the selection media, banked and frozen. The ID-secreting fusions are identified by comparing the IgV_H CDR3 sequences of the fusions with the patient's tumor. The selected hybridoma cell is expanded through multiple passages in tissue culture flasks. For antibody production, the cells are inoculated and grown in a sterile, disposable hollow fiber bioreactor unit, which provides for automated continuous perfusion and harvesting. Production continues until the required amount of ID is collected. ID is purified from filtered cell culture supernatants by Protein A affinity chromatography for IgG or immunoaffinity chromatography for IgM. The eluted product is dialyzed, sterile filtered and quantified for total protein; a prescribed amount is then

mixed in solution with sterile-filtered keyhole limpet hemocyanin (KLH) and conjugated using glutaraldehyde. The conjugation reaction is stopped chemically by the addition of glycine, and then the conjugated ID-KLH (BiovaxID™) is dialyzed against sterile saline and distributed into single-dose vials.

4. Development of BiovaxID

ID vaccines were first demonstrated to induce protection against tumors in animal models > 30 years ago. The first step in clinical ID vaccine development was to address whether the vaccine could be rendered immunogenic. This was largely a scientific question that needed to be addressed before asking the medical question of whether ID vaccines could induce meaningful clinical benefit. In the case of lymphoma ID vaccine development, it has taken 10 years to answer the scientific question in a definitive manner in Phase I and II clinical studies before proceeding to a Phase III clinical trial to address the question of clinical benefit.

4.1 Preclinical studies

In the early 1970s, Lynch and Eisen [11,12] demonstrated that active immunization with purified immunoglobulins (ID) from mineral oil-induced plasmacytomas induced ID-specific tumor resistance in mice. This phenomenon has been reproduced subsequently in a number of lymphoma, myeloma and leukemia models [13-18]. In 1987, Kaminski *et al.* [16] demonstrated that optimal immunization required conjugation to a strongly immunogenic carrier protein, such as KLH. Subsequently, Kwak *et al.* [18] demonstrated that the use of granulocyte-macrophage colony-stimulating factor (GM-CSF) as an adjuvant facilitated the induction of tumor-specific CD8⁺ T cells and enhanced the efficacy of the vaccine in a murine 38C13 lymphoma model. GM-CSF is likely to act by recruiting and promoting maturation of professional antigen-presenting cells, such as dendritic cells, which may in turn activate pathways of antigen processing that allow exogenous proteins to be presented by class I molecules [19]. Animal studies also demonstrated that idiotypic vaccination conferred protection against tumor challenge and could cause regression of established tumors. Taken together, these results provided the rationale for testing autologous tumor-derived idiotypic surface immunoglobulin as a therapeutic 'vaccine' against human B-cell NHL.

4.2 Phase I clinical trial of ID-KLH vaccine

The first human study of ID vaccination, a pilot study designed to determine whether it is possible to immunize against the ID portion of the protein, was pioneered by Kwak *et al.* [20] in patients with follicular lymphoma. Follicular lymphoma patients in minimal residual disease or complete remission after chemotherapy were immunized with subcutaneous injections of autologous purified tumor-derived immunoglobulin, conjugated to KLH. As no ID-specific immune responses were observed before the addition of an immunologic adjuvant to the first group of patients, patients (n = 9) subsequently received the entire series of immunizations with a standard emulsion adjuvant (syntex adjuvant formulation-1). In all, 41 patients were treated on this pilot study, with 41% demonstrating specific anti-ID antibody and 17% demonstrating cellular proliferative responses [21]. Of the 20 patients with residual disease following chemotherapy, 2 had complete regression of the tumor in association with development of a specific immune response. Thus, these results were important because they demonstrated that active immunization with purified autologous tumor-derived surface Ig conjugated to the immunogenic carrier KLH could induce sustained ID-specific immune responses in patients with lymphoma. Furthermore, induction of ID-specific immune responses was demonstrated in the setting of minimal tumor burden after conventional chemotherapy; however, it is important to recognize that convincing T-cell responses (CD8⁺) were not observed, and this single-arm trial was not designed to answer the question of clinical efficacy.

4.3 Phase II clinical trial of ID-KLH vaccine

Based on the preclinical observation that the addition of GM-CSF as an adjuvant to the vaccine induced tumor-specific CD8⁺ T cells [18], Bendandi *et al.* [22] conducted a Phase II clinical trial where 20 previously untreated follicular lymphoma patients were treated with autologous tumor-derived ID-KLH plus GM-CSF vaccine (in normal saline) following induction of clinical remission with a uniform chemotherapy regimen (prednisone, doxorubicin, cyclophosphamide and etoposide [PACE]). This produced a homogeneous group of patients, all in first complete remission (CR), who were given vaccine treatment in the setting of minimal residual disease. The vaccine was injected subcutaneously in 5 monthly doses starting ~ 6 months after completing chemotherapy to allow time for immunological recovery. The vaccine was well tolerated, and common adverse effects were injection site reactions such as erythema, induration and pruritus. There were no long-term adverse effects due to the vaccine.

Following vaccination, anti-KLH antibody and cellular responses were induced in all patients. Anti-ID antibody responses were induced in 15 out of 20 (75%) patients and ID-specific and/or tumor-specific CD4⁺ and CD8⁺ T-cell responses were observed in 19 out of 20 (95%) patients [22]. Importantly, significant levels of HLA class I-restricted killing of autologous tumor targets were also demonstrated in the vast majority of patients, suggesting the induction of a cytotoxic CD8⁺ T-cell response. The specificity for this response was shown by the lack of killing of non-neoplastic, normal B cells from the same patients [22]. Further characterization of anti-ID cellular immune responses on this and other trials demonstrated that the T cells specifically recognized multiple unique immunodominant epitopes within the hypervariable complementarity-determining regions, but not within the framework regions of the immunoglobulin heavy chain [23,24]. Monitoring patients for minimal residual disease showed that 8 out of 11 patients with PCR-positive t(14;18) chromosomal translocation breakpoints converted to PCR negativity in their blood immediately after completing vaccination and sustained their molecular remissions for a median of > 18 months (range: > 8 to > 32 months) [22]. Thus, these results provided the first convincing evidence of an antitumor effect of ID vaccination. Analysis of time to relapse also provided an independent indication of clinical benefit. With a median follow up of 9.2 years, median disease-free survival (DFS) was reached at 8 years, and the overall survival rate was 95% [25]. Although definitive statements cannot be made because this was not a randomized trial, the DFS appears to be superior to that of a historical prednisone, methotrexate, doxorubicin, cyclophosphamide and etoposide chemotherapy-treated control group (median DFS of ~ 2.2 years) [26].

In conclusion, this Phase II clinical trial demonstrated that tumor-specific CD8⁺ T cells, capable of killing autologous tumor cells, could be induced by ID-KLH vaccination in combination with GM-CSF [22]. It also demonstrated

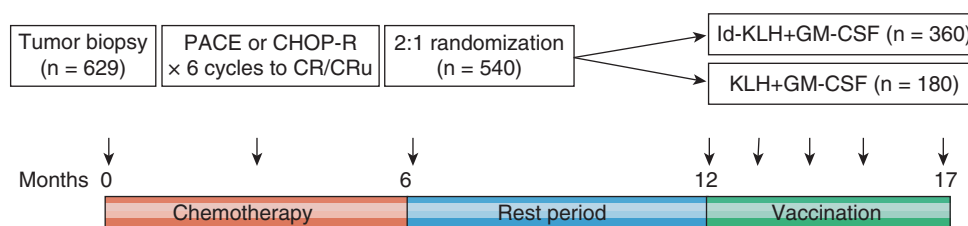


Figure 3. Treatment schema of randomized Phase III BiovaxID™ vaccine trial.

CHOP-R: Cyclophosphamide, doxorubicin, vincristine, prednisone and rituximab; CR: Complete response; CRu: Complete response unconfirmed; GM-CSF: Granulocyte-macrophage colony-stimulating factor; ID: Idiotype; KLH: Keyhole limpet hemocyanin; PACE: Prednisone, doxorubicin, cyclophosphamide and etoposide.

antitumor effects in a homogeneous group of patients and established GM-CSF as an essential component of this vaccine, as an earlier study using the same immunogen administered without GM-CSF showed humoral, but no CD8⁺ T-cell responses [20,21]. Finally, the clinical remission induced by chemotherapy may have facilitated the induction of tumor-specific immune responses by minimizing the immunosuppressive effects of bulky tumors; however, to avoid the immunosuppressive effects of the chemotherapy itself, it may be necessary to vaccinate patients following immunological recovery after chemotherapy.

4.4 ID–KLH vaccine in combination with rituximab

Understanding ID-induced tumor cell immunity has attained new clinical importance due to increased use of rituximab in the treatment of B-cell NHLs. Rituximab is an anti-CD20 monoclonal antibody that depletes both normal and malignant B cells [27,28]. Consequently, patients treated with rituximab are unlikely to have peripheral blood B cells and, therefore, are unlikely to generate humoral immune responses following active immunotherapy.

The effects of B-cell depletion induced by rituximab on immune responses to ID vaccines were recently evaluated in a pilot clinical trial in patients with mantle cell lymphoma where 26 previously untreated mantle cell lymphoma patients were treated with 6 cycles of dose-adjusted rituximab, etoposide, vincristine, doxorubicin, cyclophosphamide and prednisone, followed 12 weeks later with 5 monthly cycles of autologous tumor-derived ID–KLH plus GM-CSF vaccination [29]. Unexpectedly, despite rituximab administration, antibody responses against the carrier molecule KLH and ID were detected in 17 out of 23 (74%) and 7 out of 23 (30%) evaluable patients, respectively. The humoral responses were delayed and correlated with B-cell recovery, and were predominantly detected after the fourth or fifth vaccination, compared with after the first or second vaccinations in the follicular lymphoma study in which rituximab was not administered [22]. In contrast, vigorous CD4⁺ and CD8⁺ antitumor and KLH T-cell responses were not delayed and were induced in 20 out of 23 (87%) and 23 out of 23 (100%) patients, respectively, in the absence of circulating B cells [29].

These results indicated that ID vaccines could be administered in the setting of B-cell depletion induced by rituximab. Although the median event-free survival was only 22 months in these patients, the overall survival of 89% at 46 months raises the intriguing possibility that ID vaccination modified the natural history of mantle cell lymphoma.

4.5 Phase III clinical trials of ID–KLH vaccine

Encouraging immunological and clinical outcomes of the Phase II clinical trial that evaluated the ID–KLH plus GM-CSF vaccine in patients with follicular lymphoma led to the initiation of a randomized, double-blind, placebo-controlled, multi-centre Phase III clinical trial to definitively answer the question of clinical benefit induced by ID vaccination. This Phase III trial was initiated by the National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, and is now sponsored by Biovest International, Inc. This trial was designed similar to the Phase II trial [22] in which previously untreated advanced stage follicular lymphoma patients initially underwent an excisional lymph node biopsy and were treated into clinical remission with a PACE chemotherapy regimen. Patients who achieve a CR or complete response unconfirmed (CRu) are randomized in a 2:1 manner either to the specific vaccination arm of ID–KLH plus GM-CSF or the nonspecific vaccination arm of KLH plus GM-CSF (Figure 3). The primary endpoint for this trial is to compare DFS between the two arms [30]. The secondary objectives of this trial are to determine the ability of an ID vaccine to produce a molecular CR in patients in clinical CR by PCR evidence of minimal residual disease after standard chemotherapy; to evaluate the impact of ID vaccine to generate humoral and cellular immunologic responses against autologous tumor; and to compare the overall survival of patients randomized to receive either the autologous tumor-derived ID vaccination (ID–KLH plus GM-CSF) or nonspecific vaccination (KLH plus GM-CSF).

The Phase III trial has been recently amended and will be implemented soon to include administration of cyclophosphamide, doxorubicin, vincristine, prednisone and rituximab (CHOP-R) chemotherapy to induce remission prior to vaccine administration, based on recent results

showing that rituximab-based combination chemotherapy regimens induce a survival benefit in patients with follicular lymphoma. The trial was also amended because induction of antitumor T-cell immunity by ID vaccine was not impaired by the administration of prior rituximab-containing chemotherapy. In the amended version of the trial, 629 patients will be enrolled and 540 patients will be randomized at a 2:1 ratio of ID–KLH vaccine plus GM-CSF to KLH–KLH control vaccine plus GM-CSF. The calculated trial size is sufficient to allow > 80% power to detect a difference between DFS curves. The entire population of patients, either induced with PACE or CHOP-R, will be analyzed together for the primary efficacy analysis and, subsequently, subgroup stratifications will be examined as supportive analyses. Patients will be stratified according to the International Prognostic Index, number of chemotherapy cycles and type of chemotherapy.

Two additional randomized Phase III trials are evaluating the clinical efficacy of ID–KLH vaccine. These trials differ primarily in terms of the induction therapy and the method of ID production. The Genitope-sponsored trial uses cyclophosphamide, vincristine and prednisone chemotherapy [31], and the Favrilie-sponsored trial uses the single agent anti-CD20 monoclonal antibody rituximab [32]. Moreover, while only CR and CRu patients are vaccinated on the Biovest study, CR, CRu and PR patients are all vaccinated in the Genitope trial, and CR, CRu, PR and stable disease patients are vaccinated in the Favrilie trial. In contrast to the hybridoma method in the Biovest study, both the Genitope and Favrilie trials use recombinant DNA technology to produce the ID protein; however, the trials synthesize ID proteins with different immunoglobulin backbone structures and by different cellular systems. Of these different methods of synthesis, the ID protein in the Genitope trial is constructed with a human IgG₃ heavy chain backbone and is produced by plasmid transfection of murine lymphoma cells, whereas ID from the Favrilie trial has an IgG₁ backbone and is produced in insect cells. The results of interim analysis from these trials are expected within the next 2 years, and it will be interesting to see whether the ID vaccines can induce clinically meaningful benefit in patients with both minimal residual disease (Biovest study) as well as with low tumor burden (Genitope and Favrilie studies).

5. Role of humoral and cellular immune responses

Although some murine lymphoma models have demonstrated that the antitumor efficacy of ID vaccine depends on the induction of an antibody response [16,33,34], other lymphoma models have shown that protection against tumor challenge or eradication of established tumors requires a CD4⁺ and/or a CD8⁺ T cell response [18,35,36]. A recent analysis of 136 patients with follicular lymphoma treated with ID vaccination showed that the induction of specific anti-ID

antibody responses was associated with significantly prolonged progression-free survival (PFS) [37]. This study also demonstrated that patients with a favorable FcγRIIIa polymorphism, which predicts stronger binding of the Fc of antibodies to effector cells, had a longer PFS. Thus, patients with FcγRIIIa 158 valine/valine genotype had a longer PFS than those with valine/phenylalanine or phenylalanine/phenylalanine genotypes. In contrast, no significant correlation was observed between the development of a cellular anti-ID immune response and PFS; however, it is possible that this study may have underestimated the value of an antitumor T-cell response for several reasons. First, cellular responses were measured by a T-cell proliferation assay, which is relatively insensitive compared with newer assays, such as enzyme-linked immunospot and cytokine flow cytometry [38]. Second, the proliferation assay detects primarily a CD4⁺ T-cell response and may fail to detect a CD8⁺ T-cell response. Third, immunological assays that demonstrate recognition of native tumor (tumor-specific) may be more clinically relevant to assess T-cell responses following cancer vaccination compared with assays that demonstrate recognition of tumor antigens presented on appropriate antigen-presenting cells (antigen-specific) [39]. Finally, 86 out of 136 (63%) patients on this study received a chemical adjuvant with the ID vaccine that predominantly induced a humoral immune response [20,21]. In contrast, ID vaccine formulations using dendritic cells [40,41] or cytokines, such as GM-CSF [22,23] or IL-2 [42], as adjuvants significantly enhanced the induction of cellular immune responses.

Following ID-pulsed dendritic cell vaccination, five follicular lymphoma patients had clinical tumor regression with the induction of a T-cell response, but without an antibody response [40,41]. Similarly, following ID–KLH plus GM-CSF vaccination, three patients achieved molecular remissions without a detectable antibody response suggesting that cell-mediated antitumor immune responses are important for vaccine efficacy [22]. Clinical tumor regression was also observed in a follicular lymphoma patient after administration of a novel liposomal ID/IL-2 vaccine (OncoVAX-ID/IL-2) in the absence of an antibody response [42]. The liposomal vaccine study also showed that the tumor-specific CD4⁺ and CD8⁺ T-cell responses induced by the vaccine were sustained 18 months beyond the completion of vaccination and were associated with prolonged DFS [42]. In another study, Nelson *et al.* [43] demonstrated that the precursor frequency of tumor-specific cytotoxic T lymphocytes correlated with freedom from progression following ID vaccination. Taken together, these results suggest that T-cell responses could induce tumor regression independent of a humoral response and support the use of lymphoma vaccines in the setting of B-cell depletion induced by rituximab-based induction regimens. However, as the relative role of cellular versus humoral immunity for vaccine efficacy is uncertain, it may be advisable to administer booster vaccinations following B-cell recovery to optimize humoral responses.

6. Safety and tolerability

The side effects of the ID–KLH vaccine, such as erythema, induration and mild tenderness, have been mainly local. The vaccine or GM-CSF may cause fever, chills and flu-like symptoms. Bone pain and occasionally hypotension have been observed with GM-CSF. No long-term toxicities or deaths have been associated with the vaccine in the completed Phase II study [22].

7. Regulatory affairs

BiovaXID is being developed for commercialization by Biovest International, Inc., which holds a Cooperative Research and Development Agreement with the National Cancer Institute, National Institutes of Health (Bethesda, MD, USA). BiovaXID was recently granted Fast Track status by the FDA in the US. The FDA has also given permission to Biovest to use induction of molecular remission as a secondary endpoint in the Phase III study. The Phase III clinical trial is expected to complete accrual within the next 18 months, and analysis of the secondary endpoint on molecular remissions is ongoing.

8. Conclusion

In summary, ID vaccination appears to be safe and immunogenic in patients with NHL. The immune response appears to be directed against the tumor, but not autologous normal B cells, suggesting that ID is truly a tumor-specific antigen. Both humoral and cellular immune responses were shown to be independently associated with clinical responses following ID vaccination in patients with follicular lymphoma. Depleting normal B cells by prior rituximab administration does not seem to impair the generation of an antitumor T-cell response by ID vaccination. Single-arm Phase I and II ID vaccine trials demonstrated improved PFS compared with historical controls of patients with follicular lymphoma; however, data from ongoing randomized Phase III trials are needed to definitively determine the clinical benefit of ID vaccination in NHL.

9. Expert opinion

If successful, ID vaccines are most likely to be used as an adjuvant following standard treatment with combination chemotherapy. In addition, the recent demonstration of antitumor T-cell responses induced by ID vaccination following B-cell depletion by rituximab [29] suggests that ID vaccines can be used after administration of rituximab or rituximab-based chemotherapy. The combination of rituximab with ID vaccine would provide for the first time a combination biologic regimen for the treatment of this lymphoma. Indeed, the use of passively administered antitumor monoclonal antibodies, such as rituximab with vaccines, is likely to be complementary. Compared with

monoclonal antibodies, vaccines are likely to target different tumor antigens, and can induce immunological memory and polyclonal humoral and cellular immune responses, thereby minimizing the emergence of immune escape variants. Given that the median age of follicular lymphoma patients at diagnosis is 60 years, developing such non-toxic immunotherapeutic approaches is highly desirable.

With the increased use of rituximab for treating follicular lymphoma and other B-cell NHLs, further improvement in the potency of the ID vaccines would require strategies to enhance T-cell responses because rituximab depletes normal B cells and impairs the generation of antibody responses following vaccination. Although novel adjuvants, such as Toll-like receptor ligands, may prove to be more potent than cytokine adjuvants [44–46], further improvement of the cancer vaccines would probably also require disrupting immunoregulatory pathways that modulate the magnitude and duration of the immune response. Studies in animal models have suggested that the T-cell immune responses against foreign or self-antigens are regulated by several immunoregulatory pathways and/or peripheral tolerance mechanisms [47–49]. For example, CD4⁺CD25⁺ regulatory T cells (Tregs) have been shown to downregulate T-cell responses against foreign antigens, as well as tumor antigens, most of which are self-antigens [50]. Similarly, cytotoxic T lymphocyte-associated antigen (CTLA)-4, a molecule that is expressed on activated T cells and Tregs, was shown to downregulate T-cell responsiveness and prevent the initiation and/or limit the magnitude of autoreactive T-cell responses [51]. A host of other mechanisms, such as programmed cell death-1, B7-H1 and B7-H4, were recently described to negatively regulate T-cell responses [49]. These new insights have led several investigators to hypothesize that the potency of cancer vaccines can be further enhanced by concurrent suppression of peripheral tolerance mechanisms and/or suppressive immunoregulatory pathways. Thus, depletion of CD4⁺CD25⁺ Tregs or blockade of CTLA-4, programmed cell death-1 or B7-H1 led to improved tumor control in various murine models [52–55]. Moreover, simultaneous disruption of two immunoregulatory mechanisms by depletion of Tregs and blockade of CTLA-4 resulted in improved tumor rejection as compared with either alone when used in combination with a tumor cell-based vaccine in a B16 melanoma model [52]. These preclinical observations led to the initiation of pilot clinical trials using combination immunotherapeutic strategies, analogous to combination chemotherapy that has been effectively used for the curative treatment of certain cancers including lymphoma. Dannull *et al.* [56] have recently demonstrated that vaccine-mediated antitumor immunity is significantly enhanced in renal cell cancer patients after depletion of regulatory T cells using denileukin difitox (a recombinant IL-2 diphtheria toxin conjugate, also known as Ontak®), an FDA-approved drug for treating cutaneous T-cell lymphomas. In another study, administration of a single dose of Ontak in

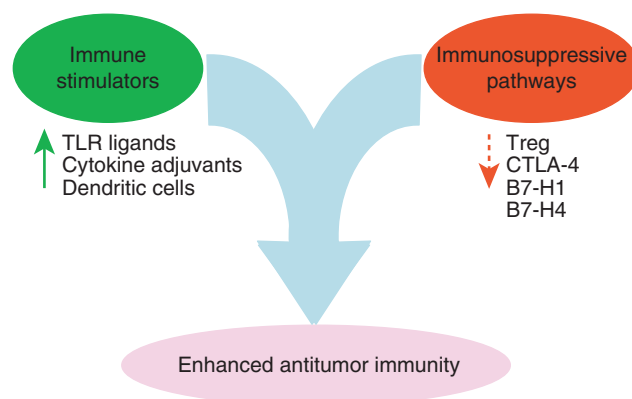


Figure 4. Factors affecting antitumor immunity. Optimal cancer vaccination strategies in the future will require the use of novel immune stimulators to enhance the potency of vaccines. In addition, simultaneous inhibition of one or more immunosuppressive pathways (eg, Tregs, CTLA-4, B7-H1, B7-H4) that limit the magnitude of antitumor T-cell responses may be necessary. CTLA: Cytotoxic T lymphocyte antigen; TLR: Toll-like receptor; Treg: Regulatory T cell.

patients with ovarian cancer depleted Tregs and was associated with enhanced endogenous immunity [57]. Similarly, blockade of CTLA-4 in combination with peptide vaccination has resulted in enhanced cancer immunity and durable objective responses in patients with metastatic melanoma [58,59]. Taken together, these preclinical and early phase clinical results support the evaluation of combination immunotherapy

strategies in future clinical trials with BiovaxID to stimulate an antitumor T-cell response and the simultaneous suppression of immunoregulatory pathways to augment the induced T-cell response (Figure 4). The existence of multiple immunoregulatory pathways necessitates systematic evaluation of these approaches in clinical trials to determine the optimal combination immunotherapy regimen.

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Affiliation

Seung-Tae Lee¹ MD, Yun-Fang Jiang² MD, PhD, Keon-Uk Park³ MD, Alison F Woo⁴ MS & Sattva S Neelapu^{†5} MD

[†]Author for correspondence

¹Postdoctoral Fellow, The University of Texas M. D. Anderson Cancer Center, Department of Lymphoma and Myeloma, Division of Cancer Medicine, 1515 Holcombe Blvd, Unit 903, Houston, TX, 77030 USA

Tel: +1 713 563 3791; Fax: +1 713 563 3424; E-mail: stlee@mdanderson.org

²Research Scientist, The University of Texas M. D. Anderson Cancer Center, Department of Lymphoma and Myeloma, Division of Cancer Medicine, 1515 Holcombe Blvd, Unit 903, Houston, TX, 77030 USA

Tel: +1 713 563 3794; Fax: +1 713 563 3424; E-mail: yjiang@mdanderson.org

³Postdoctoral Fellow, The University of Texas M. D. Anderson Cancer Center, Department of Lymphoma and Myeloma, Division of Cancer Medicine, 1515 Holcombe Blvd, Unit 903, Houston, TX, 77030 USA

Tel: +1 713 563 3791; Fax: +1 713 563 3424; E-mail: kpark@mdanderson.org

⁴Grant Program Manager, The University of Texas M. D. Anderson Cancer Center, Department of Lymphoma and Myeloma, Division of Cancer Medicine, 1515 Holcombe Blvd, Unit 903, Houston, TX, 77030 USA

Tel: +1 713 563 3432; Fax: +1 713 563 3424; E-mail: awoo@mdanderson.org

⁵Assistant Professor, The University of Texas M. D. Anderson Cancer Center, Department of Lymphoma and Myeloma, Division of Cancer Medicine, 1515 Holcombe Blvd, Unit 903, Houston, TX, 77030 USA

Tel: +1 713 563 3429; Fax: +1 713 563 3469; E-mail: sneelapu@mdanderson.org