Yonsei Medical Journal
Vol. 48, No. 1, pp. 1-10, 2007

Review Article

Therapeutic Vaccine for Lymphoma

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The unique antigenic determinants (Idiotype [Id]) of the immunoglobulin expressed on a given B-cell malignancy can serve as a tumor-specific antigen for active immunotherapy. Therapeutic vaccines targeting the tumor-specific idiotype have demonstrated promising results against lymphomas in phase I/II studies and are currently being evaluated in phase III randomized trials. Additional vaccine therapies being developed include those based on DNA, dendritic cells, gene-modified tumor cells. It is hoped that immunotherapeutic agents, used in tandem or in combination, may in the future allow effective treatment of lymphoid malignancies and delay or even replace the need for conventional cytotoxic therapies.

Key Words: Idiotype, immunotherapy, lymphoma, vaccine

INTRODUCTION

Immunotherapy has now become an important part of our therapeutic armamentarium for hematologic malignancies. Several monoclonal antibodies have been approved by the FDA and are in widespread use either alone or in combination with chemotherapy or with other biologic agents. Other passive therapies with various immune cell populations are under investigation. Active immunotherapy, whereby the host is induced to make an immune response against its own tumor cells, has long been a goal of tumor immunologists.

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 back into the patient as a therapeutic (not preventive) vaccine. The desired result is activation of both major arms of the immune response, the host antibody response and potentially a host T-cell response, against the target tumor cell or antigen, thereby aiding in the eradication of the disease.

Received December 7, 2006

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Idiotype as a tumor-specific antigen for B-cell Non-Hodgkin's lymphoma

Many of the efforts toward the development of 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, which is 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) (Fig. 1). Since B-cell NHLs are composed of clonal proliferation of mature resting and reactive lymphocytes, which express synthesized immunoglobulins on the 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.

Although not entirely applicable to Id, the major limitation to using self-tumor antigens for cancer vaccine is that they are protected by self-

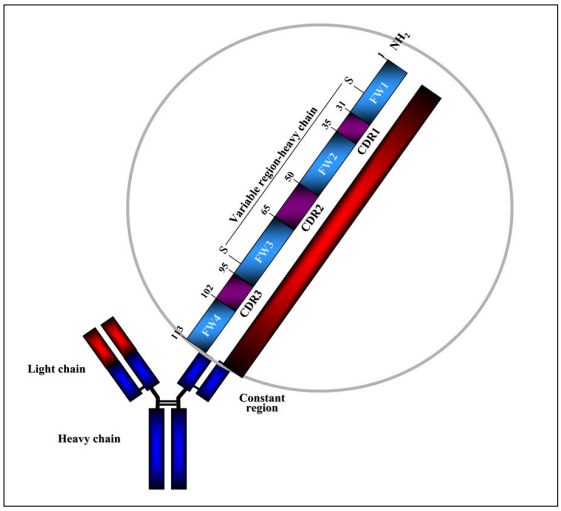

Fig. 1. Idiotype as a tumor antigen specific for B cell lymphoma. Malignancies of mature and resting B cells arise from clonal proliferation of cells that express immunoglobulins on their cell surface. Immunoglobulin molecules are composed of heavy and light chains, which possess highly specific variable regions at their amino termini and constant regions at their carboxy termini. The variable regions contain unique determinants termed idiotype (Id) that can be recognized as an antigen. The unique antigenic determinants are most likely derived from the hypervariable complementarity-determining regions (CDR), but not framework (FW) regions of the variable regions of heavy and light chains.[18] Amino acid numbers in the variable heavy chain region are shown from the amino (NH_2) terminus end.

tolerance mechanisms. The first step in cancer vaccine development is therefore to answer the question of whether it is possible to render an inherently weak tumor antigen immunogenic. This is largely a scientific question, one that should be answered in the affirmative before asking the medical question of whether cancer vaccines can induce meaningful clinical benefit. In the case of lymphoma Id vaccine development, it has taken 10 years to answer this scientific question in a definitive manner.

Preclinical studies of Id vaccine

In the early 1970s, Lynch and Eisen demonstrated in mice that active immunization with purified immunoglobulins (Id) from mineral-oil-induced plasmacytomas (MOPCs) induced Id-specific tumor resistance.[1,2] This phenomenon has been reproduced subsequently in a number of lymphoma, myeloma, and leukemia models.[3-11] In 1987, Kaminski et al., demonstrated that optimal immunization required conjugation to a strongly immunogenic carrier protein, such as KLH.[12] Subsequently, Kwak and colleagues demonstrated that the use of 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.[13] GM-CSF likely acts 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.[14] Animal studies also demonstrated that idiotypic vaccination conferred protection against tumor challenge and could cause regression of established tumor. Taken together, these results provided the rationale for testing autologous tumor-derived idiotypic surface immunoglobulin as a therapeutic "vaccine" against human B-cell NHL.

Phase I clinical trial of Id-KLH vaccine

The first human study of Id vaccination was pioneered by Kwak et al., in patients with follicular lymphoma (Table 1), which was a pilot study designed to determine whether it was possible to immunize against the Id portion of the protein.[15] 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. Because no Id-specific immune responses were observed before the addition of an immunologic adjuvant to the first group of patients, patients subsequently (nine patients) received the entire series of immunizations with a standard emulsion adjuvant (Syntex adjuvant formulation 1 - SAF-1). In all, 41 patients were treated on this pilot study; and 41% demonstrated specific anti-Id antibody and 17% demonstrated cellular proliferative responses.[15] Of the 20 patients with residual disease following chemotherapy, two patients had complete regression of the tumor in association with the development of a specific immune response. Thus, these results were important because they demonstrated that patients with lymphoma could be induced to make sustained

Id-specific immune responses by active immunization with purified autologous tumor-derived surface Ig conjugated to the immunogenic carrier KLH. Furthermore, the induction of Id-specific immune responses was demonstrated in the setting of minimal tumor burden after conventional chemotherapy.

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,[13] Bendandi and colleagues conducted a Phase II clinical trial where 20 previously untreated follicular lymphoma patients were treated with autologous tumor-derived Id-KLH+GM-CSF vaccine following induction of clinical remission with chemotherapy[16] (Table 1). 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 approximately 6 months after completing chemotherapy to allow time for immunological recovery. The vaccine was well tolerated with the main adverse effects being 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-idiotype 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.[16] 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 nonneoplastic, normal B cells from the same patients.[16] Further characterization of anti-idiotype cellular immune responses demonstrated that the T cells specifi-

Table 1. Published Clinical Trials of Idiotype Vaccination in Lymphoma

Formulation	No. of patients	Histology	Anti-Id/tumor immune response (%)		Comments	Ref
			Ab	T cell		
Id-KLH+SAF	41	FL	41	17	First human trial of Id vaccine	15
Id-KLH+GM-CSF	20	FL	75	95	Molecular remissions in 8/11 patients	16
Id-DC/Id-KLH-DC	35	FL	26	49	Clinical responses in 22% of patients	33, 34
Plasmid DNA	12	FL	0	8	Poorly immunogenic	56
Id-KLH+SAF	9	FL	89	N/A	Molecular remissions in 3/5 patients	57
Liposomal Id/IL-2	10	FL	40	100	Sustained T cell responses beyond 18 months	23
Id-KLH+GM-CSF	26	MCL	30	87	T cell responses induced in the absence of B cells	39
Id-KLH+GM-CSF	25	FL	52	72	Specific immune response associated with improved DFS	58
Fab+MF59+GM-CFS	18	FL, MM, DLBL, CLL, MCL, LPL	29	47	Specific immune response despite profound immunosuppression	59
Id-KLH+GM-CSF	31	SLL, FL	20	67	Clinical responses in 12.9% of patients	60

Id, idiotype; KLH, keyhole limpet hemocyanin; SAF, syntex adjuvant formulation; GM-CSF, Granulocyte-Macrophage Colony Stimulating Factor; DC, dendritic cell; FL, follicular lymphoma; MCL, mantle cell lymphoma; DFS, Disease-free survival; MM, multiple myeloma; DLBL, diffuse large B cell lymphoma; CLL, chronic lymphocytic leukemia; LPL, lymphoplasmacytic lymphoma; SLL, small lymphocytic lymphoma; N/A, not assessable.

cally recognized multiple unique immunodominant epitopes within the hypervariable complementarity-determining regions (CDR), but not framework regions of immunoglobulin heavy chain.[17] Monitoring of the 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).[17] 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) is 8 years, and the overall survival rate is 95%.[18] While definitive statements cannot be made, because this was not a randomized trial, the DFS appears superior to that of a historical, ProMACE chemotherapy-treated control group (median DFS, about 2.2 years).[19]

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.[17] This study also 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.[15]

Phase III clinical trials of Id-KLH vaccine

The encouraging immunological and clinical outcome of the Id-KLH + GM-CSF vaccine in the Phase II clinical trial in patients with follicular lymphoma led to the initiation of a randomized double blind placebo controlled multicenter Phase III clinical trial to definitively answer the question of clinical benefit induced by idiotype vaccination. This Phase III trial was initiated by the National Cancer Institute, National Institutes of Health, Bethesda, MD and now sponsored by Biovest International, Inc. This trial was designed similar to the Phase II trial[16] where 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 CRu (complete response unconfirmed) are randomized in a 2 : 1 manner either to the specific vaccination arm of Id-KLH+GM-CSF or the non-specific vaccination arm of KLH+GM-CSF. The primary endpoint for this trial is to compare the disease-free survival between the two arms.[20] The secondary objectives of this trial are 1) to determine the ability of Id vaccine to produce a molecular CR in patients in clinical CR but PCR evidence of minimal residual disease after standard chemotherapy; 2) to evaluate the impact of Id vaccine to generate humoral and cellular immunologic responses against autologous tumor; and 3) to compare the overall survival of patients randomized to receive either the autologous tumor-derived Id vaccination (Id-KLH + GM-CSF) or non-specific vaccination (KLH + GM-CSF).

The Phase III trial was amended in July, 2006 to include administration of CHOP-R chemotherapy for remission induction prior to vaccine administration. The rationale for this was based on the fact that rituximab-based combination chemotherapy regimens were recently shown to induce a survival benefit in patients with follicular lymphoma and due to the fact that the induction of anti-tumor T cell immunity by idiotype vaccine was not impaired by the administration of prior rituximab containing chemotherapy. In the amended version of the trial, 540 patients will be randomized in a 2 : 1 ratio of Id-KLH vaccine + GM-CSF to KLH-KLH control vaccine + GM-CSF. The calculated trial size is sufficient to allow approximately 80% power to detect a difference between disease-free survival curves with an initial hazard ratio of 1.0 for the first 8 months and then an intended hazard ratio of 2.0 thereafter. The entire population of patients, whether induced with PACE or CHOP-R, will be analyzed in a combined manner. 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 currently evaluating the clinical efficacy of Id-KLH vaccine. These trials differ primarily in terms of the induction therapy and the method of idiotype production. The Genitope-sponsored trial

uses cyclophosphamide, vincristine, and prednisone (CVP) chemotherapy,[21] and the Favrille-sponsored trial uses the single agent anti-CD20 monoclonal antibody rituximab.[22] Moreover, while only CR and CRu patients are vaccinated on the Biovest study, both CR, CRu, and PR patients are vaccinated on the Genitope trial, and CR, CRu, PR and stable disease patients are vaccinated on the Favrille trial. As opposed to the hybridoma method in the Biovest study, the Genitope and Favrille trials use recombinant DNA technology for production of idiotype protein, but with different immunoglobulin backbone structure and the cellular systems used for Id protein expression. Of these, Id protein of Genitope trial contains a human IgG3 heavy chain backbone is produced by plasmid transfection of murine lymphoma cells, whereas that of Favrille trial has IgG1 backbone and produced in insect cells. The results of interim analysis from these trials are expected within the next two years and it would be interesting to see whether the idiotype vaccines can induce clinically meaningful benefit in patients with both minimal residual disease (Biovest study) as well as with low tumor burden (Genitope and Favrille studies).

Second generation vaccines

Given that the general question of whether it is possible to immunize against Id has been answered by the completed phase II trial, and that a randomized controlled phase III clinical trial has been opened to answer the question of clinical efficacy, a third major research objective is to streamline the production of these individualized vaccines to make this therapy more practical. Accordingly, alternative methods for formulating Id, an otherwise nonimmunogenic antigen, into an immunogenic vaccine are being tested in preclinical syngeneic murine lymphoma models.

Liposomal idiotype vaccines

A strategy of replacing KLH with a more uniform liposomal carrier, containing dimyxistoyl phosphatidyl choline (DMPC) lipid and recombinant human interleukin (IL)-2 was explored and revealed that this liposomal carrier reproducibly converted lymphoma Id into a tumor rejection antigen.[23] Head to head comparisons against Id-KLH (as well as Id- KLH + GM-CSF), controlled for Id antigen dose, revealed equivalent to superior potency for the liposomal vaccine. On the basis of these results, a pilot clinical trial of this formulation was performed and the results demonstrated that liposomal delivery of Id was well tolerated and induced sustained tumor-specific $CD4^+$ and CD8+ T-cell responses in lymphoma patients.[24]

DNA vaccines

One of the major drawbacks of Id protein vaccines is that the vaccine production is time consuming and laborious. The idiotype for these protein vaccines is generated by hybridoma tissue culture technology (Fig. 2). An alternative to idiotype protein vaccination is to use DNA vaccines. Any delivery system that does not require protein expression holds tremendous potential for the goal of streamlining vaccine production. Immunoglobulin variable genes specific for the B-cell malignancies can be readily cloned[25,26] and combined into single chain variable fragment (scFv) format, encoding a single polypeptide consisting solely of VH and VL genes linked together inframe by a short, 15 amino acid linker. Preliminary studies in mice and humans showed that the DNA vaccine is weakly immunogenic in most cases and needs to

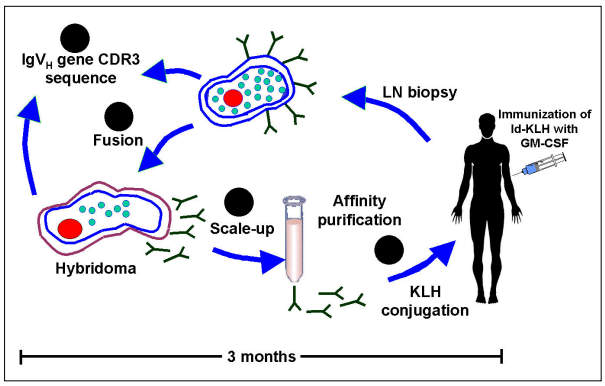

Fig. 2. Schematic diagram showing the production of Id protein vaccine using hybridoma technology. Id vaccines are custom-made from each patient's own tumor cells by fusion to the immortal myeloma cells. The Id protein is then chemically linked to the foreign protein KLH, combined with an immune-adjuvant and injected under the skin. IgV_H, immunoglobulin heavy chain variable region; CDR3, complementarity-determining region 3.

be used together with an adjuvant to render it immunogenic. King et al. demonstrated that fusion of the gene encoding fragment C of tetanus toxin to scFv markedly enhances the anti-idiotypic antibody response and induced protection against B-cell lymphoma in mice.[27,28] Biragyn and colleagues showed that the efficiency of DNA vaccination *in vivo* could be greatly increased by encoding a fusion protein consisting of idiotype (scFv) fused to a proinflammatory chemokine moiety that facilitates targeting of antigen-presenting cells for chemokine receptor-mediated binding, uptake, and processing of scFv antigen for subsequent presentation to $CD4^+$ or $CD8^+$ T cells, or both.[29-31] Specifically, mice immunized by gene gun with plasmids encoding monocyte chemotactic protein 3 (MCP-3) or interferon inducible protein 10 (IP-10)-scFv fusions, but not scFv alone, induced protective antitumor immunity against a large tumor challenge (20 times the minimum lethal dose). Furthermore, the level of protection was equivalent or superior to that of the prototype Id-KLH protein vaccine.

Cell based therapeutic vaccine approach

Dendritic cell vaccines

In the past several years, DCs have been identified as the most powerful professional APCs. Dendritic cells can take up, process, and present antigen in the context of co-stimulatory signals required for activation of both $CD4^+$ and $CD8^+$ T cells. In recent years, several strategies have been developed to exploit the antigen-presenting properties of DCs. Timmerman et al demonstrated in a murine lymphoma model that vaccination with Id-KLH-pulsed DCs induced superior tumor-protective immunity than did native Id-pulsed DCs.[32] In a pilot study, Hsu and colleagues used autologous DCs pulsed *ex vivo* with tumor-specific idiotype protein as a vaccine in 4 follicular B-cell lymphoma patients.[33] Subsequent clinical trial on 35 patients with follicular lymphoma treated with the same strategy showed a 22% overall clinical response.[34] This study demonstrated the feasibility and safety of Id-pulsed DCs as a vaccination strategy in humans.

Tumor cell vaccines

Immunization with irradiated, GM-CSF-transduced tumor cells can elicit cell mediated immunity against tumor antigens released by dying tumor cells and thereby resist growth of non-transfected tumor cells. Once again, GM-CSF serves to activate local antigen-presenting cells to efficiently take up and present these antigens to T-cells. In phase I/II studies of this approach in melanoma, renal cell carcinoma, and lung cancer, occasional clinical responses have been seen.[35-37] Levitsky et al showed that immunization of mice with lymphoma cells genetically engineered to produce GM-CSF, and to a lesser extent cells producing IL-4, eradicated pre-established systemic lymphoma.[38] The therapeutic effect of the GM-CSF- or IL-4-transfected lymphoma cells required both $CD4^+$ and $CD8^+$ T cells. In addition, the T-cell responses were shown to be Id specific in these mice, suggesting that GM-CSF-transduced tumor cell-based vaccination can induce immune responses against a native tumor antigen.

Conclusions and future prospects

Idiotype vaccination appears to be safe and immunogenic in patients with non-Hodgkin's lymphoma. The immune response appears to be directed against the tumor but not autologous normal B cells suggesting that idiotype is a truly tumor-specific antigen. Both humoral and cellular immune responses were shown to be independently associated with clinical responses following idiotype vaccination in patients with follicular lymphoma. Single arm Phase I and II idiotype vaccine trials demonstrated improved progression free survival compared with historical controls in patients with follicular lymphoma. However, data from ongoing randomized Phase III trials are needed to definitively determine the clinical benefit of idiotype vaccination in non-Hodgkin's lymphoma. If successful, idiotype vaccines are most likely to be used as an adjuvant following standard treatment with combination chemotherapy. Additionally, the recent demonstration of induction of antitumor T-cell responses by idiotype vaccination following B-cell depletion induced by rituximab[39] suggests that idiotype vaccines can be used after administration of

rituximab or rituximab-based chemotherapy. The combination of rituximab with idiotype vaccine would provide for the first time a combination biologic regimen for the treatment of this lymphoma. Indeed, the use of passively administered anti-tumor monoclonal antibodies such as rituximab with vaccines is likely to be complementary. Compared with monoclonal antibodies, vaccines are likely to target different tumor antigens, can induce immunological memory, and can induce 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, the development of such nontoxic immunotherapeutic approaches is highly desirable.

With the increased use of rituximab for the treatment of follicular lymphoma and other B-cell non-Hodgkin's lymphomas, further improvement in the potency of the idiotype vaccines would require strategies to enhance the T-cell responses since 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,[40-42] further improvement of the cancer vaccines would probably also require disruption of the immunoregulatory pathways that modulate the magnitude and duration of the immune response. Studies in animal models suggest that the T-cell immune responses against foreign or self-antigens are regulated by several immunoregulatory pathways and/or peripheral tolerance mechanisms.[43-45] For example, $CD4^+CD25^+$ regulatory T cells (T_{regs}) have been shown to downregulate T-cell responses against foreign antigens as well as tumor antigens, most of which are self-antigens.[46] Similarly, cytotoxic T lymphocyte-associated antigen (CTLA)-4, a molecule that is expressed on activated T cells and T_{regs}, was shown to downregulate T-cell responsiveness and prevent the initiation and/or limit the magnitude of autoreactive T-cell responses.[47] A host of other mechanisms such as programmed cell death 1 (PD-1), B7-H1, B7-H4 were recently described to negatively regulate T-cell responses.[45] These new insights have led several investigators to hypothesize that the potency of cancer vaccines can be further enhanced by concurrent suppression or blocking of peripheral tolerance mechanisms and/or suppressive immunoregulatory pathways. Thus, depletion of CD4+CD25+ T_{regs} or blockade of CTLA-4, PD-1 or B7-H1 led to improved tumor control in various murine models.[48-51] Moreover, simultaneous disruption of two immunoregulatory mechanisms by depletion of T_{regs} and blockade of CTLA-4 resulted in improved tumor rejection as compared with either one alone when used in combination with a tumor cell-based vaccine in a B16 melanoma model.[51] 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 and colleagues have recently demonstrated that vaccine-mediated antitumor immunity is significantly enhanced in renal cell cancer patients after depletion of regulatory T cells using denileukin diftitox[52] (a recombinant IL-2 diphtheria toxin conjugate; also known as Ontak); an FDA-approved drug for the treatment of cutaneous T-cell lymphomas. In another study, administration of a single dose of Ontak in ovarian cancer patients depleted T_{regs} and was associated with enhanced endogenous immunity.[53] 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.[54,55] Taken together, these preclinical and early phase clinical results support the evaluation of combination immunotherapy strategies in future clinical trials with idiotype vaccination for B cell lymphoma to stimulate an antitumor T-cell response and the simultaneous suppression of immune regulatory pathways to augment the induced T-cell response. The existence of multiple immune regulatory pathways necessitates systematic evaluation of these approaches in clinical trials to determine the optimal combination immunotherapy regimen.

ACKNOWLEDGEMENTS

The authors thank Allison F. Woo for review of

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