A novel proteoliposomal vaccine induces antitumor immunity against follicular lymphoma

Sattva S. Neelapu,1 Barry L. Gause,2 Linda Harvey,3 Seung-Tae Lee,1 Andrea Robin Frye,2 Jessie Horton,2 Richard J. Robb,4 Mircea C. Popescu,1 and Larry W. Kwak1

1Department of Lymphoma and Myeloma, The University of Texas M. D. Anderson Cancer Center, Houston, TX; 2Experimental and Transplantation Immunology Branch, Center for Cancer Research, National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, MD; 3SAIC, National Cancer Institute (NCI), Frederick, MD; 4XEME Biopharma, Inc., Plainsboro, NJ

Clinical studies suggest that treatment with vaccines comprised of idiotype protein may be associated with improved clinical outcome in follicular lymphoma patients. The time-consuming process required to generate patient-specific vaccines is a major limitation, however. Here we report results of a pilot clinical trial with a novel autologous, tumor-derived proteoliposome vaccine formulation that could be rapidly produced within a single day. Vaccination was safe, induced autologous tumor-specific type 1 cytokine responses in 5 out of 10 follicular lymphoma patients, and was associated with induction of a sustained complete response in one patient. Other patients had large tumor burdens and progressed after a median duration of 8 months. These results suggest that further testing of this vaccine formulation, particularly in the setting of minimal disease, is warranted. Furthermore, the proteoliposome formulation may provide a model for vaccine development for other human cancers, for which tumor-associated antigens need not be defined. (Blood. 2007;109: 5160-5163)

© 2007 by The American Society of Hematology

Introduction

Therapeutic vaccination with the unique determinants of the variable regions of the clonal tumor immunoglobulin molecule, termed idiotype (Id), induces humoral and cellular immune responses and is associated with prolonged progression-free survival in patients with follicular lymphoma.1-6 The production of Id protein by hybridoma or recombinant DNA technology is expensive and labor-intensive, however, requiring up to 6 months to manufacture the vaccine for each individual patient.7 We therefore developed a novel vaccine formulation where membrane proteins were directly extracted from autologous tumor cells and incorporated into liposomes along with IL-2 to produce membrane-patched proteoliposomes. Reports in the literature indicate that the antigen encapsulated in liposomes is delivered into both the endosomal and cytosolic processing pathways of antigen presenting cells, thereby generating both CD4+ and CD8+ T cell responses.8,9 IL-2 was chosen as a vaccine component due to its ability to expand activated T cells. Furthermore, we have previously demonstrated that IL-2 has a specific interaction with small unilamellar lipid vesicles leading to the formation of multilamellar coalescent vesicles used for vaccines.10 Testing in a mouse lymphoma model showed this formulation to be at least as potent as the prototype Id protein vaccine in inducing tumor protection (see accompanying Brief Report, Popescu MC et al). Here we report the results of our pilot clinical trial to evaluate the safety, feasibility, and immunogenicity of this novel vaccine formulation in patients with Stage III and IV follicular lymphoma.

Patients and methods

Patients

After obtaining signed informed consent, eleven previously untreated or treated patients with Stage III or IV follicular lymphoma grade 1 or grade 2 were enrolled in this National Cancer Institute institutional review board-approved Phase I clinical trial (Table 1). All patients underwent a lymph node biopsy prior to starting treatment to obtain tissue for vaccine production. Clinical responses were assessed by physical examination, computerized tomography (CT) scans, and bilateral bone marrow biopsies according to the non-Hodgkin lymphoma International Workshop Criteria.11

Vaccine formulation and administration

Proteins from whole cell membranes were directly extracted from 2 × 10⁹ lymph node biopsy cells with detergent. The membrane proteins were incorporated into liposomes along with IL-2 to produce membrane-patched proteoliposome (Oncospect-L) vaccine. Each vaccine was formulated on a per milliliter basis with membrane proteins obtained from approximately 1.6 × 10⁶ biopsy cells, 4 × 10⁶ IU of IL-2, and 80 mg of dimyristoylphosphatidylcholine (DMPC), which was used to generate liposomes. Idiotype and total protein dose were assayed in each vaccine preparation and were observed to be fairly uniform. The mean idiotype concentration in the vaccine was 2.37 µg/mL (standard deviation ± 1.02 µg/mL). The mean total protein concentration in the vaccine was 464 µg/mL (standard deviation ± 89 µg/mL). The vaccine was injected subcutaneously at 2 separate sites, either in arms or legs, at a dose of 0.5 mL per site for a total of 5 doses of the vaccine at months 0, 1, 2, 3, and 4. Two patients (UPN 9 and 11) received half the dose of each vaccine subcutaneously and the other half intratumorally in an enlarged inguinal lymph node. Patients who received
prior chemotherapy were immunized at least 6 months after the completion of the chemotherapy, to allow time for immunological recovery. Patients 7, 10, and 11 did not complete the 5 vaccinations due to progression of disease.

### Immunological assays

Cytokine induction, interferon-γ (IFN-γ) enzyme-linked immunospot (ELISPOT), and Granzyme B ELISPOT assays were performed in prevaccine and postvaccine peripheral blood mononuclear cell (PBMC) samples in parallel as previously described.5,12-14 Immune responses could not be assessed in patient 2 due to the unavailability of tumor cells.

A positive response for cytokine induction assay was defined as a response 2 or more times greater than the negative controls, which included prevaccine PBMC alone, tumor cells alone, prevaccine PBMC alone and response 2 or more times greater than the negative controls, which included prevaccine PBMC alone, tumor cells alone, prevaccine PBMC alone and response 2 or more times greater than the negative controls. Immune responses could not be assessed in patient 2 due to the unavailability of tumor cells.

A positive response for cytokine induction assay was defined as a response 2 or more times greater than the negative controls, which included prevaccine PBMC alone, tumor cells alone, prevaccine PBMC alone and response 2 or more times greater than the negative controls. Immune responses could not be assessed in patient 2 due to the unavailability of tumor cells.

A positive response for cytokine induction assay was defined as a response 2 or more times greater than the negative controls, which included prevaccine PBMC alone, tumor cells alone, prevaccine PBMC alone and response 2 or more times greater than the negative controls. Immune responses could not be assessed in patient 2 due to the unavailability of tumor cells.

A positive response for cytokine induction assay was defined as a response 2 or more times greater than the negative controls, which included prevaccine PBMC alone, tumor cells alone, prevaccine PBMC alone and response 2 or more times greater than the negative controls. Immune responses could not be assessed in patient 2 due to the unavailability of tumor cells.

A positive response for cytokine induction assay was defined as a response 2 or more times greater than the negative controls, which included prevaccine PBMC alone, tumor cells alone, prevaccine PBMC alone and response 2 or more times greater than the negative controls. Immune responses could not be assessed in patient 2 due to the unavailability of tumor cells.

A positive response for cytokine induction assay was defined as a response 2 or more times greater than the negative controls, which included prevaccine PBMC alone, tumor cells alone, prevaccine PBMC alone and response 2 or more times greater than the negative controls. Immune responses could not be assessed in patient 2 due to the unavailability of tumor cells.

A positive response for cytokine induction assay was defined as a response 2 or more times greater than the negative controls, which included prevaccine PBMC alone, tumor cells alone, prevaccine PBMC alone and response 2 or more times greater than the negative controls. Immune responses could not be assessed in patient 2 due to the unavailability of tumor cells.

A positive response for cytokine induction assay was defined as a response 2 or more times greater than the negative controls, which included prevaccine PBMC alone, tumor cells alone, prevaccine PBMC alone and response 2 or more times greater than the negative controls. Immune responses could not be assessed in patient 2 due to the unavailability of tumor cells.

A positive response for cytokine induction assay was defined as a response 2 or more times greater than the negative controls, which included prevaccine PBMC alone, tumor cells alone, prevaccine PBMC alone and response 2 or more times greater than the negative controls. Immune responses could not be assessed in patient 2 due to the unavailability of tumor cells.
of total membrane proteins as antigenic material in the vaccine formulation, we did not observe any clinical or laboratory evidence of autoimmunity in any patient on this trial. The absence of reactivity against normal B cells (Figure 1F) suggests that the T-cell responses may have been directed against tumor-associated antigens that were uniquely expressed in the lymphoma cells such as the idiotype (Figure 1H). However, it is also possible that antitumor immune responses may have been induced against tumor antigens other than idiotype with this vaccine formulation. The complete response observed in patient 5 and the prolonged stabilization of disease observed in patient 1 are unlikely due to IL-2, due to the fact that both these patients had evidence of induction of a strong anti-tumor T-cell response following vaccination (Figures 1A-C, F), and continued regression of the tumors in patient 5 was observed several months after completion of the vaccination (Figure II), suggesting the induction of a sustained anti-tumor T-cell response. These results suggest that additional testing of this formulation may be warranted, particularly in the setting of low tumor burden or minimal residual disease.

Although this novel vaccine formulation requires the generation of a custom-made product for each patient, it offers several advantages over patient-specific idiotype vaccines. First, this formulation can be produced rapidly within a single day, in contrast to the 2 to 6 months required to manufacture idiotype vaccine for each patient. Second, in addition to the membrane idiotype protein, this vaccine formulation may induce immune responses against other unrecognized tumor-associated antigens. Finally, this novel formulation may serve as a model for vaccine development against other human malignancies including certain leukemias, lymphomas, and solid tumors where tumor-associated antigens have not been defined.

Acknowledgments

We thank the physicians, pharmacy, and nursing staff of the 13E unit in Building 10, NIH Clinical Center, for their patient care. We thank A. Malyguine, S. Strobl, and K. Shafer-Weaver for performing the ELISPOT assays and Amgen for generously providing the sCD40L. We also thank the patients for participating in this trial. We thank Jessie Horton and Miriam Ferraro for help with data management and Biomira USA Inc. for manufacturing the vaccine. We thank Alison Woo for editorial assistance in preparation of the manuscript.

This work was supported by cooperative research and development agreement with Biomira USA Inc, Cranbury, NJ. This publication has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. NO1-CO-12 400.

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services.
nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Authorship


Conflict of interest disclosure: MCP, RJR, and LWK have declared a financial interest in XEME Biopharma, Inc., whose potential products were studied in the present work.

Correspondence: Sattva S. Neelapu, MD, 1515 Holcombe Blvd, Unit 903, M. D. Anderson Cancer Center, Houston, TX 77030; E-mail: sneelapu@mdanderson.org

References

A novel proteoliposomal vaccine induces antitumor immunity against follicular lymphoma