Form Approved Through 05/2004

OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed 56-character length restrictions, including spaces.

LEAVE BLANK—FOR PHS USE ONLY.		
Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT
Analysis of a Canarypox Vaccine Expressing CEA and B7

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ☒ YES
(If "Yes," state number and title)
Number: PAR-97-006 Title: Small Grants for Therapeutic Clinical Trials of Malignancies

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR

New Investigator ☒ **No** ☐ **Yes**

3a. NAME *(Last, first, middle)*
Westcott, Thomas K.

3b. DEGREE(S)
M.D.

3c. POSITION TITLE
Assistant Professor

3d. MAILING ADDRESS *(Street, city, state, zip code)*
Niels Bohr College of Medicine
1111 Central Aveneu
New York, NY 10461

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Surgery

3f. MAJOR SUBDIVISION

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: 718-430-1492 FAX: 718-430-1066

E-MAIL ADDRESS: Westcott@NBCOM.yu.edu

4. HUMAN SUBJECTS RESEARCH
☐ No
☒ Yes

4a. Research Exempt ☐ No ☐ Yes
If "Yes," Exemption No.

4b. Human Subjects Assurance No.
M106

4c. NIH-defined Phase III Clinical Trial
☒ No ☐ Yes

5. VERTEBRATE ANIMALS ☐ No ☐ Yes

5a. If "Yes," IACUC approval Date

5b. Animal welfare assurance no

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
07/01/98	06/30/00	$50,000		$100,000	

9. APPLICANT ORGANIZATION
Name Niels Bohr College of Medicine
Address 1111 Central Ave.
New York
New York

Institutional Profile File Number (if known) 01

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☐ State ☐ Local
Private: → ☒ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
162422
DUNS NO. *(if available)*
Congressional District 16

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name Mr. Emanuel Goen
Title Associate Dean for Business Affairs
Address Niels Bohr College of Medicine
1300 Central Ave.
N.Y., N.Y.
10461
Tel 718-594-1707 FAX 718-594-1812
E-Mail egoen@nbcom.yu.edu

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name Dominick P. Purpura, MD
Title Dean
Address Niels Bohr College of Medicine
1300 Central Ave.
N.Y., N.Y.
10461
Tel 718-594-1708 FAX 718-594-8613
E-Mail dpurpur@nbcom.yu.edu

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PI/PD NAMED IN 3a.
(In ink. "Per" signature not acceptable.)
Thomas K. Westcott

DATE
05/05/98

15. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13.
(In ink. "Per" signature not acceptable.)
Dominick Purpura

DATE
05/12/98

DESCRIPTION. State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely he research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This description is meant o serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, is is, will become public information. Therefore, do not include proprietary/confidential information. DO NOT EXCEED THE SPACE PROVIDED.

Nearly 500,000 patients are diagnosed annually with solid tumors that express carcinoembryonic antigen (CEA). Recent studies suggest that CEA may be a useful target for vaccine development and could, thus, benefit a large number of cancer patients. However, CEA is a self-antigen and avoiding or breaking tolerance may be required for effective anti-tumor immunity. Activation of T-cells requires both the interaction of a peptide-MHC complex with the corresponding T-cell receptor and the interaction of co-stimulatory molecules on antigen-presenting cells (APCs) with the appropriate T-cell ligand. The goal of this project is to evaluate the clinical and immunological effects of a recombinant canarypox virus (ALVAC) expressing human carcinoembryonic antigen (CEA) and the co-stimulatory molecule B7-1 in patients with advanced CEA-expressing tumors. The addition of B7-1 to the vaccine is predicted to enhance the generation of CEA-specific T-cell responses and thus break tolerance to the weakly immunogenic CEA. The optimum tolerated dose, clinical toxicity, and anti-tumor activity of the vaccine will be determined in a dose escalation phase I clinical trial.

Since patients in this trial will have advanced disease and the effectiveness of a vaccine may be limited, the patients will be evaluated for evidence of humoral and cellular immune responses as proof of vaccination. Evaluation of anti-CEA immunity will include serum CEA and cytokine levels, anti-CEA and anti-viral antibody titers by standard ELISA assays. Cellular immunity will be determined by using an intracellular interferon-γ assay or, alternatively, by ELISPOT or in vitro stimulation assays to determine the change in CEA-reactive precursor frequency T-cells through the course of multiple vaccinations in individual patients. The phenotype of reactive T-cells will be determined and long-term cultures established. The results of this project should provide insights into the immunologic and clinical effects of this new vaccine and guide future strategies for the application of tumor vaccines.

ERFORMANCE SITE(S) *(organization, city, state)*

Niels Bohr College of Medicine, NY. NY

EY PERSONNEL. See instructions on Page 11. *Use continuation pages as needed* to provide the required information in the format shown below.

lame	Organization	Role on Project
Thomas K. Westcott, M.D.	NBCOM	Principal Investigator
Heidi Ho, Ph.D.	NBCOM	Post-Doctoral Fellow
TBA	NBCOM	Research Technician
Susan Gatti, R.N.	NBCOM	Clinical Nurse
Jeffrey Abrahamson, Ph.D.	NCI	Consultant

Type the name of the principal investigator/program director at the top of each printed page and each continuation page. (For type specifications, see instructions on page 6.)

RESEARCH GRANT

TABLE OF CONTENTS

*Type density and type size of the entire application must conform to limits provided in instructions on page 6.

Appendix *(Five collated sets. No page numbering necessary for Appendix.)*

[X] Check if Appendix is included

Number of publications and manuscripts accepted or submitted for publication *(not to exceed 10)* 5

Other items *(list):*

Clinical Protocol

Principal Investigator/Program Director (Last, first, middle): Westcott, Thomas K.

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD

DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD (from page 4)	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits* *Applicant organization only*						
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES						
TRAVEL						
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		0	0	0	0	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	INDIRECT					
TOTAL DIRECT COSTS		50,000	50,000	0	0	0

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* → $100,000

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

PROJECT PERSONNEL

DR. Tom Westcott (P.I., 20% effort), will be responsible for the overall scientific direction of this project and will supervise the other key personnel involved in the project. He will also see the patients, prepare and administer the vaccine, and assist in the critical immune assays, such as the ELISPOT. Dr. Westcott has spent over four years conducting basic science research related to recombinant vaccines and tumor immunology at the NCI. He has worked closely with both Dr. Jeffrey Abrahamin the Laboratory of Tumor Immunology and Biology (LTIB) and Dr. Steven Bergstein in the Surgery Branch.

DR. HEIDI HORMEL (Postdoctoral Fellow, 50% effort), received her Ph. D. degree from the University of Wein in 1993. She then became a postdoctoral fellow for Dr. Stanley Michaelson at the Niels Bohr College of Medicine. She has extensive experience with T-cell culture and peptide preparation with respect to antigen presentation. She will be responsible for most of the immune assays performed on patient blood samples including ELISA assays, lymphoproliferation assays, cell immortalization, peptide preparation, chromium and cytokine release assays, ELISPOT, T-cell tissue culture, and T-cell cloning.

SUSAN GANT (Research Nurse, 20% effort), will be responsible for assisting in the general intake and evaluation of patients for the clinical trial. She will also assist with record keeping, data management, and specimen collection. While she will spend 20% effort on this trial, no salary support is being requested since her hospital salary already supports her use in this capacity.

BUDGET JUSTIFICATION (Continued)

TBA (Research Technician, 50% effort), will be recruited to perform T-cell cultures and other cellular immune assays. He/she will be responsible for most of the immune assays performed on patient blood samples including ELISA assays, lymphoproliferation assays, cell immortalization, peptide preparation, chromium and cytokine release assays, ELISPOT, T-cell tissue culture, and T-cell cloning. The technician will be directly supervised by a current post-doctoral fellow in the laboratory who is skilled in these techniques. The PI will oversee the entire project.

COLLABORATOR

DR. JEFFREY Abraham, Ast. Dir., LTIB. NCI. Dr. Abraham is the A.D. of the LTIB at the NCI where he has developed a recombinant vaccine program and coordinates multiple clinical trials and immune monitoring studies. His lab has developed the vaccines intended for use in this study and has experience with all of the pertinent laboratory assays proposed in this application. He will provide all necessary reagents, supplies, and technical support that may be required for the completion of these assays.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Thomas K. Westcott M.D.	Assistant Professor of Surgery, Medical Oncology Microbiology and Immunology

EDUCATION (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.*)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Illinois, Chicago, IL	B.A.	1982	Psychology/Chemistry
Loyola University, Maywood, IL	M.D.	1986	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last year three years exceeds two pages, select the most pertinent publications. DO NOT EXCEED TWO PAGES.

POSTDOCTORAL TRAINING

1986 - 1987	Internship, Department of Surgery, University of Illinois Hospital, Chicago, IL
1987 - 1988	Internship, Department of Internal Medicine, St. Francis Hospital, Evanston, IL
1988 - 1990	Medical Staff Fellowship, Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, MD
1990 - 1995	Residency, Department of Surgery, Boston University, Boston, MA
1995 - 1997	Surgical Oncology Fellowship, Surgery Branch, National Cancer Institute, Bethesda, MD

ACADEMIC APPOINTMENTS

1992 - 1995	Instructor of Surgery and Research Associate, Department of Surgery, Boston University School of Medicine, Boston, MA
1997 - Present	Assistant Professor of Surgery, Medical Oncology, Microbiology and Immunology, Mt. Sinai School of Medicine, N.Y. NY

CERTIFICATION AND HONORS

Summa cum laude, 1982; Edward J. James Scholar, 1982;
Phi Kappa Phi Honor Society, 1982; Book of University Honors, 1982;
Alpha Omega Alpha Medical Honor Society, 1986; American Board of Medical Examiners, 1987;
Daland Award, New England Cancer Society, 1992; Lester F. Williams Award, 1995;
Clinical LRP Award, NIH, 1995; American Board of Surgery, 1996; Who's Who in Health and Medicine, 1996;
Miriam Mandel Scholar, 1997

PROFESSIONAL COMMITTEES

1993 - 1994	Publications Committee, New England Journal of Medicine
1997 - Present	Biological Response Modifier Committee, ECOG

PUBLICATIONS

Dr. Westcott lists 12 references from 1991 to 1998 (date of grant application submission) mainly on CEA and viral-delivery of vaccines; journals are cancer and surgical research titles

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Hormel, Heidi, E., Ph.D.	Research Associate

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Schongauer Gymnasium, Breisach (D)	BA	1982	Biology/ English
Liebig-University of Giessen (D)	MA	1988	Nutritional Sciences
Liebig-University of Giessen (D)	MA	1989	Applied Biochemistry
University of Basel (CH) andWien (A)	Ph.D.	1994	Natural Sciences

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. DO NOT EXCEED TWO PAGES.

Postdoctoral Training

1994-Present — Research Associate, Microbiology and Immunology Department
Albert Einstein College of Medicine

Fellowships

1995-Present — Cancer Research Institute, New York, NY; Postdoctoral Fellowship

Invited Speaker

1995 — International Symposium 'Clinical Effects of Growth Hormone and Glutamine', Basel (CH)

1997 — Ludwig Institute for Cancer Research, Lausanne (CH)

Publications:

Wachs GC, Juretic A, Schultz-Thater E, Dellabona P, Filgueira L, [illegible] H, Zuber M, Garotta G, Heberer M. On the relative roles of interleukin-2 and interleukin-10 in the generation of lymphokine activated killer cell activity. Cellular Immunol. 146, 391-405, 1993.

Babst R, Hormel H, Stehle P, Brand O, Filgueira L, Marti W, Fischer M, Oberholzer M, Gudat F, Fürst P, Heberer M. Glutamine peptide-supplemented long-term total parenteral nutrition: Effects on intracellular and extracellular amino acid patterns, nitrogen economy, and tissue morphology in growing rats. J. Parenteral Enteral Nutr. 17:566-574, 1993.

Hormel H, Spagnoli GC, Filgueira L, Babst R, Gallati H, Harder F, Juretic A, Heberer M. Glutamine requirement is confined to late events of T cell activation. J. Cell. Biochem. 53:343-51, 1993.

PUBLICATION, (cont.)

Dr. Hormel lists 9 more publications in Nutrition and Clinical Immunology journals; 2 of these as first author, the rest as a middle author.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Susan Grant	Clinical Research Nurse

EDUCATION (*Begin with baccalaureate or other initial professional education. such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Purdue University, W. Lafayette, Indiana	AAS	1972	Nursing
Fordham University, New York, NY	BA	1984	Psychology
Lehman college, CUNY, Bronx, NY	MSN	1992	Clinical Nurse Specialist

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last year three years exceeds two pages, select the most pertinent publications. DO NOT EXCEED TWO PAGES.

WORK EXPERIENCE

August 1972-January 1977	Cincinnati General Hospital, Cincinnati, Ohio
January 1977-July 1979	St. George Hospital, Cincinnati, Ohio
July 1979-July 1980	Valley Hospital, Ridgewood, New Jersey
July 1980-January 1982	Mt. Sinai Hospital, New York, NY
January 1982-April 1985	St. Vincent's Hospital, New York, NY
April 1985-September 1986	St. Agnes Hospital, White Plains, NY
September 1986-November 1990	Montefiore Medical Center, N.Y., NY
November 1990-January 1992	Steri-Pharm, Home Infusion Company, Hawthorne, NY
October 1993-May 1996	Olsten Kimberly Quality Care Home Care Agency
September 1996-Present	Montefiore Medical Center, N.Y., NY

CERTIFICATION AND HONORS

Oncology Nurse Certification (OCN)	1998-2000
Certified Registered Nurse Intravenous (CRNI)	1995-1998

PROFESSIONAL COMMITTEES

1992-Present	Intravenous Nursing Society
1989-Present	American Society of Clinical Oncology
1985-Present	Oncology Nursing Society
1980-Present	Sigma Theta Tau-Honor Society of Nursing

PROFESSIONAL REGISTRATION -- New York State

Westcott, Thomas K.

PUBLICATIONS

Wiernik, P.H.; Dutcher, J.P.; Gucalp, R.; Ganti S.; and Benson, L. "MOAD Therapy in Adult Patients with Acute Lymphocytic Leukemia", Leukemia, vol. 51, pp. 58-64, July 1988.

Ganti S. "Introduction to PDQ-Physician Data Query", Hudson Valley Insider, vol. 3, number 3, December 1989.

Wiernik, P.H.; Dutcher, J.P.; Paietta, E.; Gucalp, R.: Markus, s.; Weinberg, V.; Azar, C.; Ganti S.; Benson, L. "Long Term Follow-up of Treatment and Potential Cure of Adult Acute Lymphocytic Leukemia with MOAD: A Non-Anthracycline Containing Regimen". Leukemia, vol. 7, no.8, pp. 1296-1241, August 1993.

Mazurek, C.; Dutcher, J.P.; Schwartz, E.; Ganti S.; Benson L.; Wiernik, P.H. "Phase I Clinical and pharmacokinetics Study of Menogaril (7-con-o-methylnogarol) in Previously Treated Patients with Acute Leukemia", Investigational New Drugs, vol. 11 pp. 313-328, November 1993.

RESEARCH PLAN

Specific Aims

1. To determine the degree of host immunity against CEA after vaccination with an ALVAC-CEA-B7 vaccine by in vitro analyses of T-cell reactivity and antibody titers.

   This study represents the first attempt to vaccinate human patients with advanced CEA-expressing tumors using a recombinant canarypox virus (ALVAC) that expresses both human CEA and the B7-1 co-stimulatory molecule. We hypothesize that multiple vaccinations with this vaccine will result in enhanced CEA-specific immune responses. Since we plan to conduct a phase I clinical trial, only patients with advanced, metastatic disease will be evaluated. This is a particularly difficult patient population to treat since tumors are well established and patients often immunosuppressed. Thus, if the vaccine is to be developed for other patient populations, such as those with minimal residual disease or at high risk for tumor development, parameters other than clinical outcome are necessary. We propose to show that the vaccine does induce both humoral and cellular immune responses by *in vitro* analysis of T-cell reactivity and anti-CEA antibody titers after vaccination with the vaccine. T-cell assays will be accomplished using a novel assay that measures intracellular cytokine release after antigen stimulation, which may prove to be a more sensitive assay and easier to use for monitoring vaccine patients. We will also perform standard ELISPOT and stimulation assays for evaluation of T-cell responses. Standard ELISA will be used to determine antibody titers. Results from this study should help clarify the immune response of cancer patients to the ALVAC-CEA-B7 vaccine and may elucidate a new method for monitoring cancer vaccines in future trials with this or other vaccines.

2. To determine the optimum tolerated dose, toxicity, and clinical effect of an ALVAC-CEA-B7 vaccine through the conduct of a controlled phase I clinical trial.

   This study proposes a dose escalation clinical trial design using a recombinant ALVAC-CEA-B7 vaccine. Animal data suggests that higher doses of ALVAC may be more effective at generating CTL responses. We hypothesize that specific anti-CEA immune responses will be enhanced with increasing doses of vaccine with minimal toxicity. The study will enroll 6 patients at three increasing dose levels of vaccine ($4.5 \times 10^6 - 4.5 \times 10^7 - 4.5 \times 10^8$ PFU). Patients will be monitored for side effects and clinical responses. Results from this study will determine the optimum tolerated dose, pharmacologic toxicity, and clinical effectiveness of this new vaccine. Correlation with anti-CEA immune responses will also be evaluated and will guide future vaccine clinical trials with this agent.

Background and Significance

Carcinoembyonic antigen is a 180,000 dalton glycoprotein that is characterized by an oncofetal pattern of developmental expression (1). Normal adult colonic epithelium expresses low levels of CEA, whereas most CEA-expressing cancer cells express much higher levels. Detection of CEA in the circulating peripheral blood of cancer patients is a useful marker for recurrent disease (2). CEA is expressed by a variety of tumor types, including nearly all colon and rectal cancers, many gastric, pancreatic, and ovarian cancers, 50% of breast cancers, and 70% of non-small cell lung cancers (3). The gene encoding CEA is located on chromosome 19 and has been isolated and cloned (4). The

protein backbone contains a leader sequence and three highly conserved internal repeat domains consisting of 178 amino acids each (5).

CEA was first isolated in 1965 by Gold and Freedman (6). Shortly after that time there was interest in the immunogenicity of CEA in cancer patients. Early reports focused on the development of antibodies against CEA with some evidence that increased titers were present in colon cancer patients (7). Further studies suggested that circulating immune complexes, composed of CEA bound to antibody, were present in cancer patients and these complexes could interfere with serum antibody determination (8). There are fewer reports of cellular immune responses to CEA in the literature. However, recent investigations have identified two T-cell specific epitopes in CEA using a computer generated program based on the consensus binding motifs of random CEA peptide sequences to the HLA-A2 molecule (9). These sequences predicted two 9-mer peptides from within the internal repeat domain region of CEA and were designated CEA-associated peptide-1 (CAP-1) representing amino acid sequence $CEA_{571\text{-}579}$, and CEA-associated peptide-2 (CAP-2), representing amino acid sequences $CEA_{555\text{-}564}$. These peptides were able to elicit specific CTL activity from peripheral blood lymphocytes derived from patients vaccinated with a vaccinia-CEA vaccine (10). This renewed interest in using CEA as a target for vaccine immunotherapy of CEA-expressing cancers.

The HLA-A2-restricted CAP peptides represent class I-restricted epitopes that generate $CD8^+$ CTL responses as measured by *in vitro* assays, consistent with other reports with A2-restricted peptides (10,11). *In vitro* assays often require multiple rounds of stimulation with peptide before functional activity can be detected (12). Furthermore, in a recent melanoma clinical trial, a modified HLA-A2-restricted gp100 peptide administered with interleukin-2 (IL-2) resulted in a 42% response rate. However, the clinical responses did not correlate with *in vitro* assays of T-cell reactivity to the class I restricted modified peptide (13). The reasons for this paradox are unclear and it is possible that the currently employed assays do not accurately reflect *in vivo* activity of the peptides. Repetitive stimulation *in vitro* may artificially induce responses in T-cells that would not normally be activated *in vivo*. Another possibility is that a single class I restricted peptide is not sufficient for therapeutic immunity. This notion is supported by recent reports that $CD4^+$ T-cells are required for antigen-specific CTL generation (14). Other investigators have also been able to isolate $CD4^+$ T-cell epitopes from known tumor antigens (15). The actual *in vivo* presentation of potential epitopes from CEA has not been studied and could provide evidence that other epitopes or multiple epitopes are more appropriate as targets for CEA-directed vaccines. Insertion of the full-length CEA cDNA in the viral genome should lead to presentation of all potential CEA epitopes after immunization.

While evidence suggests that human T-cells recognize specific epitopes derived from CEA peptide sequences, to date there have been no documented anti-tumor clinical responses using such vaccines (16). One possible explanation for this is that vaccines presenting only antigen, such as CEA, to the immune system may be ineffective at generating adequate T-cell activation. Activation of antigen-specific T-cells requires two separate signals, one from the interaction of the MHC-peptide complex with the corresponding T-cell receptor (TCR) and the other from ligation of co-stimulatory molecules expressed on APC with their T-cell ligand. Effective vaccine strategies will likely also require that two separate signals are delivered to effector T-cells after vaccination. The most widely studied co-stimulatory system is the binding of the B7-1(CD80) and B7-2(CD86) molecules to the T-cell surface ligands, CD28 and CTLA-4, respectively. The murine and human B7-1 molecules have been cloned

and the cDNAs inserted into vaccinia virus genomes. In a murine CEA-tumor model, an admixture of recombinant vaccinia viruses expressing CEA and murine B7-1, resulted in more effective tumor treatment than either virus used alone (17). Similar results were also obtained using an admixture of vaccinia viruses expressing murine B7-1 and a modified MUC-1 gene (18). Chamberlain et al. reported a reduction in pulmonary metastases using a β-galactosidase-expressing tumor in mice treated with a single recombinant vaccinia virus expressing both *LacZ* and the murine B7-1 gene (19). Clinical trials in human patients using recombinant poxviruses expressing both human CEA and B7-1 have not been performed prior to this study.

ALVAC is a canarypox virus that has the ability to infect human cells, express foreign gene sequences for 2-3 weeks, and does not replicate or result in lytic infection of host cells. ALVAC has been used extensively in veterinary vaccines and has been used for vaccination against rabies and HIV in clinical trials. Preclinical studies in mice demonstrated that a recombinant ALVAC-CEA virus elicits specific anti-CEA CTL and results in therapeutic effectiveness against CEA-expressing murine tumors. Potential advantages using ALVAC as a vector include: 1.) safety, due to the non-replicative nature of ALVAC in non-avian systems, and; 2.) the ability to more efficiently elicit an immune response to the foreign immunogens compared to vaccinia vectors. This is though to be due to the strong anti-viral neutralizing antibody responses that occur after immunization with vaccinia virus, making it difficult to provide adequate booster doses for cell-mediated immunity against weak immunogens, such as CEA. GMP-grade ALVAC-CEA has been manufactured by the MegapharMAceutical Corporation and approved by the FDA for phase I testing.

Since many new anti-cancer vaccines are first used in humans through the conduct of a phase I clinical trial, the first patients exposed to such vaccines often have quite advanced disease. These patients represent a particularly difficult population to use for determination of clinical responses. The use of vaccines may be more appropriate for patients with earlier stage disease, minimal residual disease, or those at high risk for future disease. In order to apply current vaccine candidates to such patients, we need to show some evidence that the vaccine can induce immunity, even if there are no clinical responses. However, the best method for monitoring vaccine patients is not well defined. Measurement of anti-CEA serum antibody titers can easily be performed, although animal data suggests that T-cell immunity is a better measure of effective vaccination (20). T-cell responses can be evaluated using simple proliferation assays or more specific assays, such as the ELISPOT assay (21). Although it is often difficult to isolate CTL, this can sometimes be accomplished with evidence of increased CTL after vaccination (10). These assays are often difficult to perform because of poor yield of T-cells, low precursor frequency rates, and high background. New assays that allow for easy and sensitive predictors of adequate immunization are needed to guide therapeutic strategies and identify potential vaccines for further clinical development.

Preliminary Studies

We have previously conducted a clinical trial using a vaccinia virus expressing human CEA (rV-CEA) in patients with advanced colorectal cancers (16). These patients were monitored by ELISPOT assay using peripheral blood mononuclear cells before and after vaccination. Analysis of immune responses from these patients revealed a post-vaccination increase in the precursor frequency of CEA-reactive T-cells by interferon-γ ELISPOT assay (see Figure 1).

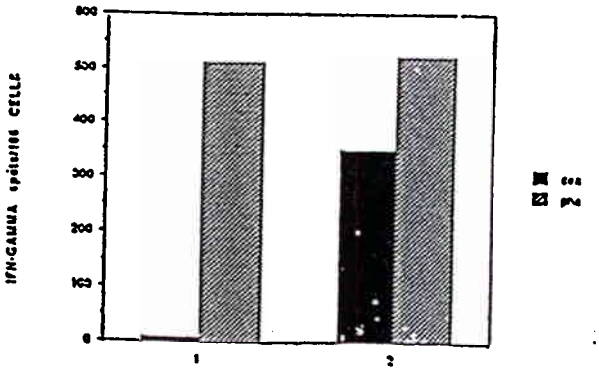

Figure 1. Representative ELISPOT assay result from one Patient before (1) and after (2) vaccination with the rV-CEA vaccine. An increased precursor frequency of CEA-Reactive T cells can be seen by increased IFN-γ release After T-cells were cultures with patient-derived dendritic Cells pulsed with whole protein CEA (darkened bars). T-Cell viability was confirmed by pulsing with the mitogen PHA (hatched bars).

Although the ELISPOT assay reveals evidence of increased anti-CEA immunity following vaccination, the assay is cumbersome, laborious to reproduce, and has a high background making analyses difficult to interpret. We have, therefore, attempted to use an assay that measures intracellular cytokine expression from whole blood. This assay is easier to perform directly from collected peripheral blood samples and may be more sensitive than the ELISPOT (22).

In order to establish the intracellular cytokine assay, whole blood from two normal donors was collected. 500 µl of whole blood was diluted with RPMI tissue culture medium for analysis. The cells were activated with Phorbol 12-Myristate 13 Acetate (PMA), a T-cell mitogen, and Ionomycin (I), a necessary co-factor for PMA activity. Intracellular cytokine expression was detected after incubation with brefeldin A (BFA), a drug that disrupts Golgi-mediated transport and allows newly synthesized cytokines to accumulate. This yields an enhanced cytokine signal that can be detected by flow cytometry. In general, cells were incubated for 4 to 6 hours at 37°, 7% CO_2 and then labeled with fluorescent antibodies for detection. A second sample of cells was used as controls without mitogenic activation.

First, the lymphocyte population was characterized according to size and granularity features (FSC and SSC) among the whole blood cell population and defined as region 1 (R1). All subsequent analyses were restricted to this cell population (see Fig. 2).

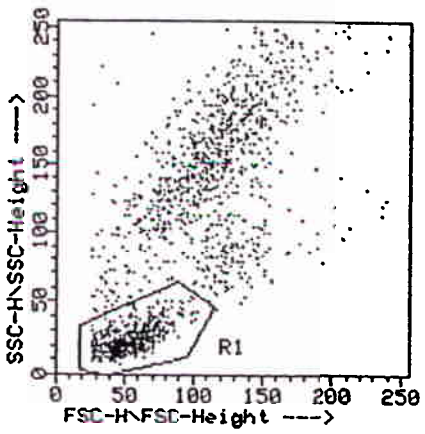

Figure 2. Dot-plot illustration of a FACS analysis from whole blood taken from a normal volunteer. The plot shows the cell size by forward scatter (x-axis) and the cell granularity by side scatter (y-axis). The gated cells represent the lymphocyte population (R1).

Initially, further evaluation was restricted to $CD4^+$ lymphocytes, which were characterized by the upregulation of CD69 and their intracellular expression of IFN-γ. In the unstimulated control group there was no increased expression of CD69 or IFN-γ production (Fig. 3, A and B). In the PMA and I stimulated sample, $CD4^+$ T cells were activated as shown by upregulation of the early activation marker CD 69 (Fig. 3, C) and increased expression of intracellular IFN-γ (Fig. 3, D).

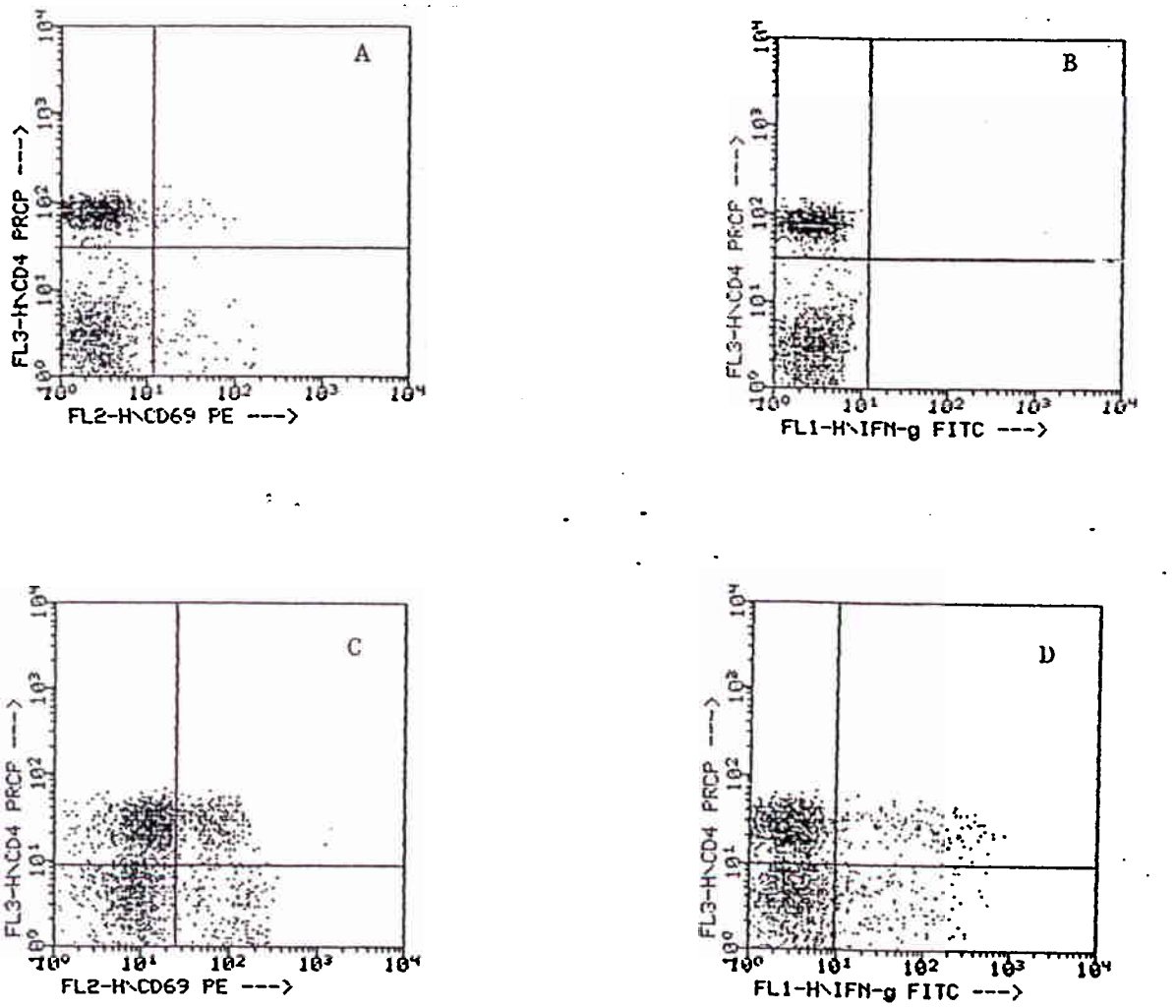

Figure 3. Dot-plot illustration of FACS analysis from the same volunteer showing that the lymphocytes (gated from R1 in figure 2) increase CD69 and intracellular IFN-γ levels after mitogenic stimulation. These plots show selected $CD4^+$ T-cells by anti-$CD4^+$ $antibody_{PerCP}$ (y-axis) against CD69 expression determined by anti-CD69 $antibody_{PE}$ (x-axis) for unstimulated (A) or stimulated (C) cells. The same cell population was tested for intracellular IFN-γ expression using an anti-IFN-γ $antibody_{FITC}$ (x-axis) for unstimulated (B) and stimulated (D) cells.

These studies have shown that the intracellular cytokine staining method can be accomplished using whole blood samples. We plan to extend these assays to our vaccinated patients using CEA-stimulated T-lymphocytes as a technique for monitoring the immune response following immunization.

Research Design and Methods

Aim 1: To determine the degree of host immunity against CEA after vaccination with an ALVAC-CEA-B7 vaccine by in vitro analyses of T-cell reactivity and antibody titers.

Intracellular Cytokine Staining Assay

Since T-cell reactivity against CEA may be the most important parameter of enhanced anti-tumor immunity in vaccinated patients, we plan to perform selected T-cell assays as a measure of immune responsiveness to the recombinant vaccine. We hypothesize that after a series of three immunizations with the ALVAC-CEA-B7 vaccine there will be an increase in CEA-specific T-cell responses. The optimal method for assessment of these responses is unknown and we propose to evaluate a new technique based on the intracellular production of interferon-γ from activated T-cells. This method should be more sensitive and easier to perform than other standard techniques, such as the ELISPOT assay.

Activation of discrete subsets of T cells result in typical patterns of cytokine release. For example, $CD4^+$ Th_1 cells release IL-2, TNF-α, and IFN-γ, whereas Th_2 cells secrete IL-4, IL-5, IL-6, and IL-10 (23). In order to evaluate antigen-specific responses of distinct T cell subsets following administration of the ALVAC-B7-CEA-vaccine, we plant to measure intracellular expression of cytokines after incubation with CEA peptide stimulation and brefeldin-A (BFA). BFA disrupts intracellular Golgi-mediated transport and allows cytokines to accumulate, yielding an enhanced cytokine signal that can be detected by flow cytometry. This unique method can detect multiple cytokines per cell and discrete cellular populations that express a particular cytokine (22).

Whole blood will be collected in sodium heparin VACUTAINER tubes (BectonDickinson) and diluted 1:1 in RPMI-1640 without serum (1ml total volume). Cyropreserved PBMC resuspended in medium (RPMI-1640, 10% FCS) can also be used for the assay. Samples will be activated in the presence of BFA (10μg/ml cell suspension). The activated control sample will be performed using Phorbol 12-Myristate 1113 Acetate (PMA) and Ionomycin (I) (Sigma) at 25ng/ml and 1μg/ml final concentration, respectively. The activated sample will be stimulated with the CEA derived HLA-A2 restricted peptide epitope CAP-1, $CEA_{571-579}$, at final concentrations ranging from 2-5 μM. The unstimulated control sample will also contain BFA. All incubations will be performed in 12 x 75-mm capped polystyrene test tubes (Falcon) and incubated for at least 4-5 hrs at 37°C, 7% CO_2. Then, for cell surface staining, 100μl of each sample will be stained with 10μl of each mAb, $CD3_{PerCP}$, CD4 or $CD8_{PerCP}$, $CD69_{PE}$, control IgG_1/G_{2a} (all BectonDickinson), as indicated. After 15 min of incubation in the dark at RT, samples will be lysed by adding 1 ml of 1X FACS lysing solution (BectonDickinson) and further incubated for 10 min. After centrifugation for 5 min at 500 x g, the supernatant will be discarded and then 1 ml of FACS permeabilizing solution (BectonDickinson) will

be added. After 10 min of incubation, samples will be washed 2-3 times in wash buffer (PBS with 0.5% BSA and 0.1% NaN_3) and centrifuged for 5 min at 500 x g. After discarding the supernatant, intracellular staining will be determined by adding 10µl of anti-IFN-γ mAb $_{FITC}$ or an isotype control IgG_1/G_{2a}. After 30 min of incubation and washing with 2 ml of wash buffer, samples will be centrifuged for 5 min at 500 x g, resuspended in 500µl of fixation medium (PAF 0.2%) and stored at 4°C in the dark until FACS acquisition.

Data Management, Pitfalls, and Alternatives

The intracellular cytokine detection may be used to detect approximate numbers of specific T cells even when present at low frequencies. However, we might be unable to induce cytokine production in some patients probably due to various levels of immunosuppression or due to low cell numbers. As Kabilan et al. showed, there is a qualitatively good correlation between the intracellular immunofluorescence staining and ELISA detection of cytokines (24). Thus, we will also measure serum cytokine levels as discussed below. If the intracellular cytokine staining method is not successful, we propose several alternative assays for determination of T-cell immunity against CEA. This includes the IFN-γ ELISPOT assay and standard T-cell stimulation and proliferation assays.

ELISPOT Assay

Cells secreting IFN-γ in an antigen-specific manner can be detected using a standard ELISPOT assay (25,26). Briefly, 96-well polyvinylidene difluoride backed plates (MAIP S45: Millipore, Bedford, MA) are coated with 10µg/ml of anti- IFN-γ mAb 1-D1K (Mabtech, Stockholm, Sweden) overnight at 4°C. Plates are washed 6 times with RPMI-1640 and blocked with RPMI-1640 supplemented with 2mM L-glutamine, 100U/ml penicillin, 100µg/ml streptomycin, and 10% heat-inactivated pooled human AB serum (complete medium) for 1 hr. Cyropreserved PBMC are thawed into complete medium (CM), washed once and resuspended at a 5x 10^6/ml. PBMC are added in 100µl/well to the precoated plates. Adherent PBMCs are used as antigen-presenting cells (APC) after pulsing with the CEA peptide (CAP-1) at a final concentration of 2µM. The release of IFN-γ in PBMC stimulated with ConA mitogen (Sigma) is included as a positive control. Plates are incubated for 20 hrs at 37°C, 5%CO_2, then stopped by shaking off the contents and washing 6 times with PBS 0.05% Tween 20 (Sigma). 100µl of 1µg/ml of the biotinylated anti-IFN-γ mAb, 7-B6-1 (Mabtech), is added and after 3 hrs of incubation, plates are washed 6 times and a 1:1000 dilution of strepavidin alkaline phosphatase conjugate (Mabtech). After 2 hrs of incubation at RT, plates are washed 6 times and 100µl chromogenic alkaline phosphatase substrate (BioRad Labs., Hercules, CA), diluted 1:25 in deionized water, is added to the wells. After 30 min, the colorimetric reaction is terminated by washing with tap water and plates are air dried.

IFN-γ SFC (spot-forming cells) are enumerated as previously described (26). Spots are counted under 20X magnification using a stereomicroscope. Only large spots with fuzzy borders are scored as SFCs as per standard convention. Responses are considered significant if a minimum of five SFCs are present per well, and additionally, this number is at least twice that of negative control wells.

Data Management, Pitfalls, and Alternatives

Methods to determine antigen-specific T cells are generally based on the measurement of their target-specific cytotoxicity, as in the standard chromium release assay, on their precursor frequencies (limiting dilution assay), or on specific proliferation or cytokine release upon recognition of their target. Using the ELISPOT technique enables the measurement of cytokine release from a single cell allowing direct calculation of T-cell frequencies (27). ELISPOT assays have been shown to detect and quantitate peptide-specific T cells among peripheral blood lymphocytes without the need for prior *in vitro* expansion (28,29). The ELISPOT assay has also been reported to correlate closely with the level of cytotoxicity after 14 days of *in vitro* expansion (29). We have previously used the technique to monitor vaccine patients with good results. However, the ELISPOT is a cumbersome assay and failure to obtain adequate T-cell recovery, especially from cryopreserved samples, has often limited the ability to complete this assay.

In Vitro Stimulation Assay

Cyropreserved PBMC's will be thawed in to complete medium (CM), consisting of Iscove's modified DMEM with 25mM Hepes buffer (Gibco), 10% heat-inactivated pooled human AB serum (Bioreclamation, Inc.), 2mM l-glutamine, 100U/ml penicillin, 100µg/ml streptomycin (Gibco). Cells will be washed once and resuspended at 1.5 x 10^6 cells/ml in 2ml containing 2µM CEA peptide (CAP-1). Two days later recombinant human IL-2 (300 IU/ ml) will be added to the cultures. On day 5, CM (1ml) will be withdrawn and replaced with fresh CM containing IL-2. CM (1ml) will be replaced whenever the medium becomes acidic. Cells will be harvested between days 11 and 13 after initiation of cultures. The harvested cells will be washed once in HBSS and 10^5 cells will be added in 0.1ml to wells of flat bottom 96-well plates (Costar). Stimulator cells, T2 cells (TAP-deficient, HLA-A2-expressing cells) pulsed with peptide, will be added in 0.1 ml. To pulse T2 cells with CAP-1 peptide, either 10^{-4} µM, 10^{-2} µM or 1µM peptide will be incubated with 6 x 10^6 T2 cells in 3ml for 3 hrs at 37°C with intermittent mixing. The cells will be washed once with HBSS before addition to the responder cells. Cultures will be incubated for 18-24 hrs at 37°C in 5% CO_2. IFN-γ release into the supernatant will be measured using a standard ELISA assay (Endogen). Positive wells will be defined as >2 times IFN-γ release compared to wells stimulated with unpulsed T2 cells.

Data Management, Pitfalls, and Alternatives

This assessment of immune reactivity of PBMCs is based on the ability to generate specific anti-CEA peptide reactivity following *in vitro* exposure to immunizing peptide. Rosenberg et al. demonstrated that this *in vitro* sensitization assay was highly specific (13). PBMCs obtained from patients following two injections of the melanoma peptide $gp100_{209\text{-}2M}$ in IFA could be specifically sensitized *in vitro* to the native $gp100_{209\text{-}217}$ peptide and correlated with the reactivity against two HLA-A2 melanoma cell lines.

If the *in vitro* stimulation assay is not successful, cell proliferation will be monitored by the standard ^{3}H-thymidine incorporation measuring 'de novo' synthesis of DNA. This will be performed using standard techniques, as described elsewhere (30).

Serum Cytokine and CEA Levels

Serum CEA and IFN-γ levels will be measured using standard ELISA assays according to the manufacturer's protocol (Endogen, Inc.). Other cytokines will also be measured, including IL-4, IL-10, IL-12, and TGF-β using standard ELISA assays on serum collected from patients before and after vaccination.

Data Management, Pitfalls, and Alternatives

ELISA assays have become standard laboratory practice with reproducible results and acceptable sensitivity.

Antibody Responses

The humoral response to vaccination with the ALVAC-CEA-B7 vaccine will be assessed by standard ELISA assays (31). Briefly, patient sera obtained on specific days of treatment will be analyzed for the presence of antibodies against CEA protein and canarypox virus. Briefly, plates will be coated either with dried down purified CEA protein or alternatively with GEO cells expressing CEA protein; or plates will be coated with irradiated canarypox virus. To prevent non-specific Abs from binding, plates will be incubated with 5% BSA in PBS for 1 hr. After extensively washing, plates will be incubated with five-fold dilutions of patient sera for 1 hr. After washing, horseradish peroxidase-conjugated sheep anti-mouse IgG $F(ab')_2$ fragments (Amersham) will be added for 1 hr at 37°C to detect anti-CEA or anti-ALVAC Abs, respectively, immobilized on the wells. The resulting complexes will be detected by the chromogen, *o*-phenylenediazamine (Sigma) and absorbance will be read at 490nm using a ELISA plate reader. As a positive control the well-characterized anti-CEA mAb, COL-1 will be used (32).

Data Management, Pitfalls, and Alternatives

One potential problem using ELISA for measurement of anti-CEA antibodies is the isolation of antibodies directed against CEA-related proteins, such as normal cross-reacting antigen (NCA), which can cross-react with CEA and produce a false-positive result. One method to circumvent this is to perform a Western blot using CEA protein (soluble or baculovirus-derived CEA, kindly provided by Dr. Jeffry Abraham). Patient sera will be run on the Western against the protein and binding with the predicted 180,000 kDa band will be indicative of anti-CEA antibodies. Binding will be detected by ECL conjugated to horseradish peroxidase (hrp), as described elsewhere (33). Briefly, the protein sample is separated by electrophoresis, transferred to a nitrocellulose membrane, and treated with sera after blocking non-specific sites. The membrane is then incubated with hrp-labeled conjugate, treated with ECL detection reagents, and exposed to film. Isotypes can be determined by incubating with biotinylated second antibody followed by exposure to a pre-formed hrp-streptavidin complex, treated with detection reagents, and exposed to film. In order to avoid interference by immune complexes the sera will be pre-heated so that only unbound antibody will be detected.

The COL-1 monoclonal antibody was generated from extracts of colon adenocarcinomas and reacts with several epitopes of human CEA (34). COL-1 exhibits higher affinity constants for CEA (13.6 x

108 M-1) compared to other anti-CEA mAbs. Cross-competition analyses established COL-1 as superior to other antibodies for identification of human tumor-derived CEA (32). We have used this antibody extensively in previous evaluation of CEA-expressing cells, viruses, and protein preparations. This should provide an adequate positive control and we expect these assays to be standard without major pitfalls.

Aim 2: To determine the optimum tolerated dose, toxicity, and clinical effects of the ALVAC-CEA-B7 vaccine through the conduct of a phase I clinical trial.

Animal experiments have shown that recombinant poxvirus vaccines expressing surrogate human tumor antigens are effective at prevention and treatment of established murine tumors expressing the same human tumor antigens (20,35). Safety of both vaccinia virus and ALVAC virus for human administration has been confirmed through several clinical trials (16,36). Recently, further support for the use of CEA as a vaccine target has been derived from the isolation of two specific peptide epitopes within the CEA protein backbone that can be recognized by CTL from patients vaccinated with a vaccinia-CEA vaccine (10). Although vaccination against CEA appears rational and safe, the lack of any therapeutic effectiveness thus far remains problematic. Although there may be several explanations for this, the lack of appropriate co-stimulation during antigen presentation may be one important reason. Previous pre-clinical investigations have shown that the addition of the B7-1 co-stimulatory molecule into the genome of a poxvirus enhanced antigen-specific CTL generation (17,19). The addition of B7-1 to vaccination strategies targeted against human tumor antigens has not been previously reported. We hypothesize that the combination of both CEA and B7-1 in a recombinant canarypox virus (ALVAC) will increase CTL generation and improve clinical effectiveness without significant toxicity.

We plan to test the above hypothesis by conducting a dose escalation phase I clinical trial using the recombinant ALVAC-CEA-B7-1 vaccine. The protocol for this study has been written and approved by the institutional review board (IRB) at the Niels Bohr College of Medicine and by the Cancer Therapy Evaluation Program (CTEP) at the National Cancer Institute (the approved protocol can be found in Appendix II). The vaccine has been manufactured by MeGaPharMa-ceutical Corporation (Rye NY) under FDA-approved guidelines and an investigational new drug (IND) application has been filed. We propose to enroll six patients in one of three escalating doses of vaccine. The first cohort of patients will receive 4.5×10^6 PFU of the vaccine by intramuscular injection at four week intervals for a total of three vaccinations. The second cohort of patients will receive 4.5×10^7 PFU, and the third cohort will receive 4.5×10^8 PFU administered in a similar fashion. These doses are based on the concentration of the vaccine as supplied by the manufacturer. Vaccine is provided in vials containing 4.5×10^8 PFU in a one milliliter solution. Patients eligible for participation include patients over 18 years old with a histologic diagnosis of adenocarcinoma expressing CEA. Patients must also have measurable disease, no other standard therapeutic options, and have no medical or immunological contraindications to study participation. The full eligibility criteria can be found in the protocol (Appendix II). This study has also been approved by the Office of Recombinant DNA Activities (NIH) and was recently granted approval by the FDA.

The clinical trial will be conducted on an out-patient basis at the Niels Bohr General Clinical Research Center (GCRC). Patients will be screened by the principal investigator and his staff, which

includes a full-time clinical research nurse, data manager, GCRC staff nurses, and a senior post-doctoral fellow. Eligible patients will be enrolled and blood collected for clinical toxicity assessment and immunological evaluation. Immune studies will involve collection of extra blood at each patient visit for serum (antibody and cytokine assays) and T-cell isolation (cellular immune assays). Clinical data will be recorded using standard clinical research protocol forms and guidelines. Data management will be performed with biostatistical support from the Niels Bohr Cancer Center. The clinical course, laboratory evaluation, and immune assay results will be reviewed on a weekly basis in order to manage patients on study as well as for reporting purposes. Follow-up imaging of all probable disease sites will be done four weeks after the final vaccination and compared to pre-treatment measurements. Parameters for clinical and radiological responses are defined in the protocol and use standard reporting criteria for clinical oncology studies.

Data Management, Pitfalls, and Alternatives

At the conclusion of this clinical trial, all collected data will be evaluated and reported for evidence of clinical toxicity and tumor responses. This information will be correlated with determinations of anti-CEA antibody titers, serum CEA levels, cytokine levels, and T-cell responses, as outlined under Aim 1 of this proposal. This information should allow an assignment of the optimum tolerated dose, which is defined as the optimal dose for generation of anti-CEA immunity (humoral and cellular), clinical anti-tumor responses (if any), and minimal or acceptable toxicity. This dose will be used in future studies with this vaccine. Standard statistical analyses will be performed in consultation with a full-time biostatistician through the Cancer Center as appropriate.

There are many potential pitfalls with any clinical trial, especially when eligible patients have advanced neoplastic disease. We will make every attempt to enroll only patients who clearly meet all eligibility criteria and are able to give informed consent. Since this vaccine has not been previously tested the full range of side effects and clinical responses are difficult to predict. However, we have not seen any major adverse events with the more pathogenic vaccinia vaccines to date. Furthermore, we will make every attempt to collect clinical data in accordance with protocol guidelines and maintain accurate records and computer data entry. The institution has a long-standing track record conducting clinical trials and has specific interests in immunotherapy protocols. We expect to enroll all 18 patients within one year of the trial start date.

Human Subjects

Human subjects are required in this proposal to test the new recombinant ALVAC-CEA-B7 vaccine. Individuals with advanced CEA-expressing adenocarcinomas over the age of 18 will be eligible provided that they meet all entry criteria. This includes measurable disease, elevated CEA expression, HLA typing, Good performance status (ECOG <2), 3 month or greater life expectancy, no major cardiac, pulmonary, hepatic, renal, or neurologic medical conditions, no immunosuppressive diseases or active infections, no contraindications to poxvirus vaccines, and ability to give informed consent. The study is designed to enroll 18 patients over a period of one year. These patients will be recruited from patients with advanced CEA-expressing tumors, including those arising from the colon, rectum, stomach, pancreas, breast, and lungs (non small cell cancers). This represents a very large patient population with roughly equivalent numbers of men and women (except breast). A large number of minority patients should also be affected with these diseases and will be eligible for participation. Patients will not be excluded based on sex, race, nationality, or ethnic composition.

Patients will be recruited from the local area for participation in this study. Collection of blood will be performed as part of the study and maintained in the investigator's laboratory. An institutional informed consent contains information regarding the proposed nature of the research, procedures involved. Potential benefits, potential risks, alternative treatments, and specific information and consent for blood samples to be collected. A copy of the consent form can be found at the end of the clinical protocol in Appendix II. The principal investigator and/or a member of the research team (approved by the local IRB) will discuss the above information with each patient and obtain informed consent PRIOR to treatment on study. Every effort will be made to recruit women and minority candidates from the local public hospital and out-patient oncology clinics. Our institution is located in N.Y. (NY) and served a varied ethnic and racial patient population. As an example, below is a chart containing the ethnic composition of the last 12 patients accrued into a clinical trial by the principal investigator of this grant (updated 5/11/98):

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown	Total
Female		2		1	4		7
Male		1		1	3		5
Unknown							
Total		3		2	7		12

The potential risks associated with this study include side effects from the vaccine or complications related to drawing blood. The expected side effects from the vaccine include pain at the injection site, muscle weakness, localized adenopathy, fever, night sweats, and other mild flu-like symptoms. These have been described in other patients receiving ALVAC vaccines before. No treatment has been required and all symptoms appear to be self-limited lasting only a few days. Complications from phlebotomy include localized pain, hematoma, and rarely, infection. Patients will have approximately 50 cc of blood drawn at each visit. There is a minimal risk of radiation from required radiological imaging studies. However, the amount of radiation exposure is minimal and these studies would most likely be necessary for the routine care of these patients, regardless of their participation in this study.

Patient records will be kept strictly confidential and patients will not be identified by name in any publication resulting from this study. The records will be available to selected officials from several institutional committees or organizations. This includes the institutional review board (IRB), National Cancer Institute (NCI), Food and Drug Administration (FDA), and the manufacturer of the vaccine (MedImmune Inc.). This will be explained to all patients before entry into the study and is explicitly written in the consent form. Every effort will be made to keep the results of any information generated during the trial confidential. If any adverse event should occur, including all potentially life-threatening event, immediate medical intervention will be available to all patients. The principal investigator is an attending physician with full hospital privileges for admission and treatment of patients. The Niels Bohr College of Medicine is a tertiary care facility with complete diagnostic and therapeutic facilities and departments for 24-hour consultation.

The potential benefit to patients enrolling in this study is prolonged survival, treatment of their cancer, improved quality of life, and the opportunity to participate in medical research that may help other cancer patients in the future. We have seen very few side effects in previous vaccine trials and thus, the likelihood of significant toxicity is low. While most patients readily agree to the trial, we will explain the realistic benefits, potential side effects, and procedures involved before obtaining consent. All patients will be given at least 24 hours to make an informed decision before signing the consent form. If there are any acceptable alternatives for a patients (i.e. radiation to symptomatic metastases, chemotherapy options, or other experimental studies) these will be explained on an individual basis. This is necessary because of the wide range of tumor types we will be evaluating (i.e. there are more options for a woman with breast cancer who has not received chemotherapy than for a pancreatic cancer patient who has completed 5-FU).

This study involves an investigational new drug (IND # 1492) and the 30 day interval between submission of applicant certification to the FDA and its response has elapsed.

VERTEBRATE ANIMALS: (None)

LITERATURE CITED: (not Reproduced Here)

pages 25, 26, 27 NOT INCLUDED

Consortium, Contractual Arrangements- None

Consultants/Collaborators

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Dr. Jeffrey Abraham will be a consultant on this grant. He will provide necessary reagents for the immunological assays to be performed, including CEA protein, CEA-associated peptides, and monoclonal antibodies (for tissue immunohistochemistry and HLA typing). Dr. Abraham will also be available to provide laboratory training for our post-doctoral fellows should that become necessary. This may be helpful for further experience in ELISPOT assays, which have been done in Dr. Abraham's laboratory.

Westcott, Thomas K.

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U.S. Department of Health and Human Services
Public Health Services
National Institutes of Health

January 5, 1998

M.D.
Assistant Professor
Departments of Surgery, Medical Oncology, and
Microbiology and Immunology
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Ronin Building, Room 204
1111 Central Avenue
N.Y. NY 10461

Dear Dr. Westcott

I am writing this letter to inform you that I am delighted to collaborate with you on the project titled "Analysis of a Canarypox Vaccine Expressing CEA and B7." The Laboratory of Tumor Immunology and Biology at the NCI has extensive experience in the area of tumor vaccines and immunological monitoring of vaccine patients. We will be happy to provide you with any reagents, supplies or technical assistance that you may need as a result of the immune assays stemming from the proposed clinical trial.

I look forward to working with you on this endeavor. Please let me know if I can be of further assistance.

Sincerely yours,

Jeffrey Abraham

Jeffrey Abraham Ph.D.
Asst. Dir. Laboratory of Tumor
Immunology and Biology
National Cancer Institute

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Jeffrey Abrahm, Ph.D.	Laboratory of Tumor Immunology and Biology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Rutgers University, New Brunswick, NJ	Ph.D.	1969	Immunology
Adelphi University, Garden City, NY	M.S.	1966	Microbiology
Ohio State University, Columbus, OH	B.S.	1964	Biology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. DO NOT EXCEED TWO PAGES.

Professional Experience:

1982 - present Chief, Laboratory of Tumor Immunology and Biology, Division of Basic Sciences, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

1973 - present Professor (Adjunct), Graduate Faculty in Genetics, George Washington University, Washington, D.C.

1980 - 1982 Chief, Experimental Oncology Section, Laboratory of Cellular and Molecular Biology, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

1976 - 1980 Head, Tumor Virus Detection Section, Laboratory of Viral Carcinogenesis, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

1973 -1976 Chairman, Breast Cancer Virus Segment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

1971 - 1973 Assistant Professor, Department of Human Genetics and Development, College of Physicians and Surgeons of Columbia University, New York, New York

1969 - 1970 Instructor, Department of Human Genetics and Development, College of Physicians and Surgeons of Columbia University, New York, New York

Publications:

Abrams S, Hodge JW, McLaughlin J, Steinberg S, Kantor J, and Abrahm J. Adoptive immunotherapy as an In Vivo model to explore antitumor mechanisms induced by a recombinant anticancer vaccine. J Immunother 20: 48-59, 1997.

Akagi J, Hodge JW, McLaughlin JP, Gritz L, Panicali D, Kufe D, Abrahm J, and Kantor J. Therapeutic antitumor response after immunization with an admixture of recombinant vaccinia viruses expressing a modified MUC1 gene and the murine T-cell costimulatory molecule B7. J Immunother 20:38-47, 1997.

Correale P, Walmsley K, Nieroda C, Zaremba S, Zhu M, Abrahm J and Tsang KY. In Vitro Generation of Human Cytotoxic T Cells Specific for Peptides Derived from Human Prostate-Specific Antigen. J Nat Cancer Inst 89:293-300, 1997.

McLaughlin JP, Abrams S, Kantor J, Abrahm J, and Greiner JW. Immunization with a syngeneic tumor infected with recombinant vaccinia virus expressing granulocyte-macrophage colony-stimulating factor (GM-CSF) induces tumor regression and long-lasting systemic immunity. J Immunother 20: 449-459, 1997.

Tsang KY, Zhu MZ, Nieroda CA, Correale P, Zaremba S, Hamilton JM, Cole D, Lam C, and Abrahm J. Phenotypic Stability of a Cytotoxic T Cell Line Directed Against an Immunodominant Epitope of Human Carcinoembryonic Antigen. Clinical Cancer Research 3: 2439-2449, 1997.

Dr. Abraham lists 18 more publications from 1991 to 1997 in Cancer Immunology and Vaccine journals. In 1991-1994, four of them are with Dr. Westcott, with the topic being vaccinia virus carrying CEA for purposes of vaccination.