Exercise 2

Reviewer's Scoring Report

**Overall Impact.** After considering all of the review criteria below, briefly summarize the significant strengths and weaknesses of the application and state the likelihood of the project to exert a sustained powerful influence on the field.

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

**Instructions:** The NIH grant application scoring system uses a 9-point scale. A score of 1 indicates an exceptionally strong application with essentially no weaknesses. A score of 9 indicates an application with serious and substantive weaknesses with very few strengths; 5 is considered an average score.

Ratings are in whole numbers only (no decimal ratings). This scale is used by all eligible (without conflict of interest) assigned reviewers to score five individual criteria (e.g., Significance, Investigator(s), Innovation, Approach, and Environment):

- Reviewers should feel free to assign the score that they believe best represents the impact of the application, and not feel constrained to limit their scores to the upper half of the score range if they do not feel such a score is warranted.
• After the session, individual reviewer scores will be averaged and the result multiplied by 10 to determine the final impact/priority score.
• The range of the final application scores is from 10 to 90.

Scoring Criteria

1. Significance. Does the project address an important problem or a critical barrier to progress in the field? If the aims of the project are achieved, how will scientific knowledge, technical capability, and/or clinical practice are improved? How will successful completion of the aims change the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field?

Circle Rating: 1 2 3 4 5 6 7 8 9

2. Investigator(s). Are the PD/PIs, collaborators, and other researchers well suited to the project? If Early Stage Investigators or New Investigators, or in the early stages of independent careers, do they have appropriate experience and training? If established, have they demonstrated an ongoing record of accomplishments that have advanced their field(s)? If the project is collaborative or multi-PD/PI, do the investigators have complementary and integrated expertise; are their leadership approach, governance and organizational structure appropriate for the project?

Circle Rating: 1 2 3 4 5 6 7 8 9

3. Innovation. Does the application challenge and seek to shift current research or clinical practice paradigms by utilizing novel theoretical concepts, approaches or methodologies, instrumentation, or interventions? Are the concepts, approaches or methodologies, instrumentation, or interventions novel to one field of research or novel in a broad sense? Is a refinement, improvement, or new application of theoretical concepts, approaches or methodologies, instrumentation, or interventions proposed?

Circle Rating: 1 2 3 4 5 6 7 8 9
4. **Approach.** Are the overall strategy, methodology, and analyses well-reasoned and appropriate to accomplish the specific aims of the project? Are potential problems, alternative strategies, and benchmarks for success presented? If the project is in the early stages of development, will the strategy establish feasibility and will particularly risky aspects be managed?

If the project involves clinical research, are the plans for 1) protection of human subjects from research risks, and 2) inclusion of minorities and members of both sexes/genders, as well as the inclusion of children, justified in terms of the scientific goals and research strategy proposed?

Circle Rating: 1 2 3 4 5 6 7 8 9

5. **Environment.** Will the scientific environment in which the work will be done contribute to the probability of success? Are the institutional support, equipment and other physical resources available to the investigators adequate for the project proposed? Will the project benefit from unique features of the scientific environment, subject populations, or collaborative arrangements?

Circle Rating: 1 2 3 4 5 6 7 8 9

**Summary of Scores:**

1. Significance: 

2. Investigator(s): 

3. Innovation: 

4. Approach: 

5. Environment: 

\[\text{Average Total Score: } \]

3 | Page
Can You Use These Samples Safely?

Through the generosity of the PIs, we can share this actual grant application, but use this information with care.

While you may find it useful to see an actual application, today's R01s are different. Don't be misled by the level of detail or section length of the samples.

R03 applications are now shorter -- 6 pages for the Research Strategy and 1 page for Specific Aims -- and have some structural differences.

Because of confidentiality issues, we expect that PIs will be able to share their applications publicly starting in 2010. At that time, we will start seeking outstanding examples for our audience.
Grant Application

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
   Number: PAR-97-006   Title: Small Grants for Therapeutic Clinical Trials of Malignancies

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR
   New Investigator □ No □ Yes
   Westcott, Thomas K.
   3b. DEGREE(S)
   M.D.
   3d. MAILING ADDRESS (Street, city, state, zip code)
   Niels Bohr College of Medicine
   1111 Central Avenue
   New York, NY 10461
   E-MAIL ADDRESS: Westcott@NBCOM.yu.edu

4. HUMAN SUBJECTS RESEARCH
   4a. Research Exempt □ No □ Yes
   If "Yes", Exemption No.
   4b. Human Subjects Assurance No.
   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)
   From 07/01/98 Through 05/30/00
   7a. Direct Cost ($) 50,000
   7b. Total Cost ($) 100,000

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

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
   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 718-594-6813
   E-Mail dpurpura@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.

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 PI/PD NAMED IN 3a.
(In ink, "Per" signature not acceptable).

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

DATE
05/06/98
05/12/98
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.
# RESEARCH GRANT

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| Resources | – |

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| Introduction to Supplemental Application (Not to exceed 1 page) | – |
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## Checklist

| Personnel Report (Compelling Continuation only) | – |

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

## Appendix

(Three to five collated sets. No page numbering necessary for Appendix.)

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

Other items (list):

| Clinical Protocol | |

PHS 398 (Rev. 5/93) (Form Page 3) Page 3

Number pages consecutively at the bottom throughout the application. Do not use suffixes such as 3a, 3b.
**BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD**
**DIRECT COSTS ONLY**

<table>
<thead>
<tr>
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**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. TROY 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 Abrahams in the Laboratory of Tumor Immunology and Biology (LTIB) and Dr. Steven Bergstein in the Surgery-Branch.

**DR. HEIDI HORN** (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 Mishell at the 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 GAMPER** (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 and 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 Abramson, Asst. Dir., NCI. Dr. Abramson is the Director 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.

POsITION TITLE

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.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE</th>
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<tbody>
<tr>
<td>University of Illinois, Chicago, IL</td>
<td>B.A.</td>
<td>1982</td>
<td>Psychology/Chemistry</td>
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<tr>
<td>Loyola University, Maywood, IL</td>
<td>M.D.</td>
<td>1986</td>
<td>Medicine</td>
</tr>
</tbody>
</table>

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 individual 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, NYC, 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

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**

Hommel, Heidi, E., Ph.D.

**POSITION TITLE**

Research Associate

**EDUCATION/TRAINING**

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

<table>
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<th>YEAR(s)</th>
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<tr>
<td>Schongauer Gymnasium, Breisach (D)</td>
<td>BA</td>
<td>1982</td>
<td>Biology/English</td>
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<tr>
<td>Liebig-University of Giessen (D)</td>
<td>MA</td>
<td>1988</td>
<td>Nutritional Sciences</td>
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<tr>
<td>Liebig-University of Giessen (D)</td>
<td>MA</td>
<td>1989</td>
<td>Applied Biochemistry</td>
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<tr>
<td>University of Basel (CH) and Wien (A)</td>
<td>Ph.D.</td>
<td>1994</td>
<td>Natural Sciences</td>
</tr>
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</table>

**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:**


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.

Susan Gant

POSITION TITLE
Clinical Research Nurse

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

<table>
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<td>Purdue University, W. Lafayette, Indiana</td>
<td>AAS</td>
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<tr>
<td>Fordham University, New York, NY</td>
<td>BA</td>
<td>1984</td>
<td>Psychology</td>
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<tr>
<td>Lehman College, CUNY, Bronx, NY</td>
<td>MSN</td>
<td>1992</td>
<td>Clinical Nurse Specialist</td>
</tr>
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</table>

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 pages 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

February 1982-April 1985
St. Vincent's Hospital, New York, NY

April 1985-September 1986
St. Agnes Hospital, White Plains, NY

September 1986-November 1990
Monteris Medical Center, NY, 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, NY, 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
PUBLICATIONS


Mazurek, C.; Dutcher, J.P.; Schwartz, E.; Gait, S.; Benson L.; Wiernik, P.H. "Phase 1 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 x 10^6 – 4.5 x 10^7 - 4.5 x 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

Carcinoembryonic 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).
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 CEA571-579, and CEA-associated peptide-2 (CAP-2), representing amino acid sequences CEA555-564. 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 ß-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 thought 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 present 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 (rVCEA) 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% CO₂ 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 (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 FVTC (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+ Th1 cells release IL-2, TNF-α, and IFN-γ, whereas Th2 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 plan 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, CEA571-579, 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% CO2. Then, for cell surface staining, 100μl of each sample will be stained with 10μl of each mAb, CD3PE, CD4 or CD8 PerCP, CD69PE, control IgG1/G2a (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% NaN₃) 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 FR1C or an isotype control IgG₁/G₂a. 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. Cryopreserved PBMC are thawed into complete medium (CM), washed once and resuspended at a 5x 10⁶/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%CO₂, 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

Cytopreserved PBMC's will be thawed in to complete medium (CM), consisting of Iscove's modified DMEM with 25mM Hapes 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% CO2. 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 gp100209-217 in IFA could be specifically sensitized in vitro to the native gp100209-217 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 3H-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-phenylenediamine (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 (hpr), 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 hpr-labeled conjugate, treated with ECL detection reagents, and exposed to film. Isotypes can be determined by incubating with biotinylated secondary antibody followed by exposure to a pre-formed hpr-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 **Immunex 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 $4.5 \times 10^6$ PFU of the vaccine by intramuscular injection at four week intervals for a total of three vaccinations. The second cohort of patients will receive $4.5 \times 10^7$ PFU, and the third cohort will receive $4.5 \times 10^8$ 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 $4.5 \times 10^8$ 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 pretreatment 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):

<table>
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<th>American Indian or Alaskan Native</th>
<th>Asian or Pacific Islander</th>
<th>Black, not of Hispanic Origin</th>
<th>Hispanic</th>
<th>White, not of Hispanic Origin</th>
<th>Other or Unknown</th>
<th>Total</th>
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<td>Female</td>
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<td>7</td>
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<td>12</td>
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</table>
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)
Consortium, Contractual Arrangements - None

Consultants/Collaborators

Jeffrey A. Abraham, Ph.D.
Laboratory of Tumor Immunology and Biology
National Cancer Institute
Building 10, Room 471
10 Center Drive
Bethesda, MD 20892

(301) 496-0001

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.
January 5, 1998

M.D.
Assistant Professor
Departments of Surgery, Medical Oncology, and
Microbiology and Immunology
Mehl Bahr College of Medicine
Ruskin Building, Room 204
111 Central Avenue
N.Y. N.Y. 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,

[Signature]

Jeffrey D. Abraham
Ph.D.
Laboratory of Tumor Immunology and Biology
National Cancer Institute
Jeffrey A., Ph.D.  Laboratory of Tumor Immunology and Biology

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

<table>
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<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE</th>
<th>YEAR(S)</th>
<th>FIELD OF STUDY</th>
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<tr>
<td>Rutgers University, New Brunswick, NJ</td>
<td>Ph.D.</td>
<td>1969</td>
<td>Immunology</td>
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<tr>
<td>Adelphi University, Garden City, NY</td>
<td>M.S.</td>
<td>1966</td>
<td>Microbiology</td>
</tr>
<tr>
<td>Ohio State University, Columbus, OH</td>
<td>B.S.</td>
<td>1964</td>
<td>Biology</td>
</tr>
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</table>

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

Publications:
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.