AD_____

GRANT NUMBER DAMD17-94-J-4403

TITLE: Poliovirus Tumor Vaccine for Breast Cancer Micro-Metastases

PRINCIPAL INVESTIGATOR: Casey D. Morrow, Ph.D.

CONTRACTING ORGANIZATION: University of Alabama at Birmingham
Birmingham, Alabama 35294-2010

REPORT DATE: September 1998

TYPE OF REPORT: Final

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1998	Final (15 Aug 94 - 14 Aug 98)

4. TITLE AND SUBTITLE
Poliovirus Tumor Vaccine for Breast Cancer Micro-Metastases

5. FUNDING NUMBERS
DAMD17-94-J-4403

6. AUTHOR(S)
Casey D. Morrow, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Alabama at Birmingham
Birmingham, Alabama 35294-2010

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

The underlying reason for failure to cure patients diagnosed with breast cancer is the presence of micrometastases. The stimulation of anti-tumor immune responses represents one of the most effective ways to treat low tumor burdens that are clinically occult. The objective of our proposal was to determine whether a poliovirus replicon vaccine strategy induces systemic immunity and eradication of micrometastases.

During our project, we have constructed and characterized poliovirus replicons which express CEA or HER2/neu. Mice given these replicons generate anti-CEA or HER2/neu antibodies. We have demonstrated protection from tumor challenge in the mice which have antibodies to CEA. The significance of these studies is that we have now characterized the appropriate replicons that will be used to establish route of administration for generating an immune response against CEA or HER2/neu prior to tumor challenge. We developed companion replicons which express biologically active immune modulators (GMCSF and IL-2) to increase the immunogenicity of the replicons encoding CEA or HER2/neu. Characterization of the immune response to antigens in mice revealed a typical TH1 response. The results of our studies provide the foundation for future strategies to use replicons as anti-tumor vaccines.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Vaccine, Mouse Model, Poliovirus, Carcino-Embryonic Antigen, Micro-Metastasis, Therapy, Breast Cancer	12
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

(n/a) Where copyrighted material is quoted, permission has been obtained to use such material.

(n/a) Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

(n) Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

(n) In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

(n) In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

(n/a) In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

(n) In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Casey D. Moore 9/7/98

PI - Signature Date

Table of Contents

Department of the Army
Final Report
Grant No. DAMD17-94-J-4403

Front Cover: 1

SF 298 Report Documentation Page: 2

Foreword: 3

Table of Contents: 4

Introduction: 5

Body: 5-9

Conclusions: 9

References: 9-10

Appendix: –

Bibliography (Final): 11

List of people supported by grant: 12

INTRODUCTION (Rational taken from proposal).

The frequency of micro-metastatic cancer deposits has correlated with a number of factors at the time of primary therapy of breast cancer including regional lymph node metastasis, size of primary tumor, tumor differentiation and certain molecular aspects of individual tumors including aneuploidy, DNA synthesis (S phase) and presence or absence of amplified gene expression for c-myc, p53, or HER2/neu. The use of systemic hormonal therapy, chemotherapy or combined adjuvant therapy directed at these micro-metastases has produced a reduction in metastatic relapse rate and improved survival. These effects are modest, and the majority of patients with micro-metastases still have disease recurrence and ultimately die of metastatic breast cancer. **Thus, it is clear that additional strategies to eradicate micro-metastases at the time of initial diagnosis is a major priority in breast cancer therapy research.** It should also be noted that our inability to detect occult micro-metastases means that patients with and without micro-metastases will be treated. The strategy should have low toxicity, ease of administration and low cost.

Multiple animal model studies (6, 7) have demonstrated that immune responses to tumor associated antigens can have dramatic antitumor effects, but such treatment strategies rapidly lose efficacy as progressive tumor growth occurs. Thus, the induction of an immune response to tumor-associated antigens in humans is likely to have limited success in patients with obvious metastases, and its optimal application would be at the time of occult micro-metastasis as an adjunct to primary therapy. Active specific immunotherapy to enhance host immune response to tumor associated antigens has been called "vaccine" therapy, although this application does not fit the strict (narrow) definition that entails prevention of disease rather than therapy of an existing disease. **It is the purpose of this project to define a novel strategy to enhance antitumor response to tumor-associated antigens, leading to the development of therapeutic vaccines.**

A variety of studies have demonstrated that immunization with tumor preparations can produce antitumor immune responses in animal models (4, 5, 12). Similar studies in man have shown antitumor effects in patients with melanoma (3, 10) and other tumors (9). The ability to isolate and clone putative tumor antigens provides the opportunity to utilize more defined reagents and to allow analysis of specific immune responses in guiding the design of active immunotherapy trials. A variety of potential targets include CEA, HER2/neu, MUC-1, MAGE 1, mutated RAS, mutated p53, etc. (7). A number of genetically engineered cancer vaccines utilizing cloned tumor associated antigens in vaccinia virus constructs or with adjuvants are undergoing clinical trials (1). This approach represents a fertile and novel new technology, since we are just beginning to identify potential tumor associated antigens (and their genes) which will be applicable to novel strategies for enhancement of anti-tumor immune responses in animal models and man.

Poliovirus Replicons to Express Foreign Genes.

The proposed experiments were based on the use of poliovirus as an expression vector for proteins to deliver antigens to immunoreactive sites of the immune system. Poliovirus is attractive for use as a vector for cancer vaccines for several reasons. First, it is an RNA virus with no DNA intermediates in replication. Thus, we can formulate vaccines with oncogenes (e.g. HER2/neu) without concern for cellular transformation (8). Second, a unique vector system based on poliovirus for the expression of foreign genes has been developed by my laboratory. We have constructed poliovirus genomes encoding foreign proteins, referred to as replicons, for over twenty different genes. We developed the procedure for complementation of these replicons by providing the capsid protein *in trans*. We are able to generate stocks of encapsidated replicons which encode foreign proteins and which, upon infection of cells, express this recombinant protein. We have demonstrated that administration of replicons alone to experimental animals results in production of an immune response to the foreign protein (11).

BODY OF THE PROPOSAL

The Specific Aims of the proposal have not changed and are as follows:

1 To construct poliovirus replicons which express native and truncated CEA proteins (including secreted and non-secreted molecules (months 1-18).

2. To characterize and optimize the immune response to CEA elicited by both oral and parenteral administration of such vaccines in mice (months 4-24).

3. To test the ability of such poliovirus - replicon CEA vaccines to generate antitumor effects as measured by resistance to tumor challenge in a syngeneic murine CEA expressing breast cancer model (months 12-36).

4. To test the therapeutic effects of such vaccines in the eradication of breast cancer micro-metastasis in a syngeneic, spontaneously metastasizing CEA positive breast cancer model (months 18-48).

We have made consistent progress towards the completion of the designated experiments described in Specific Aims 1-3. To summarize our accomplishments, we have constructed poliovirus replicons which encode several different versions of the CEA protein (2). Three different replicons were constructed and demonstrated to express CEA upon infection of cells. Based on the results of our studies, two replicons were chosen for further analysis. The first replicon contains the complete CEA gene minus the signal sequence (CEA sig-). The second replicon contains the CEA gene lacking the sequences that specify transmembrane anchoring (CEA-TM-). Both of these replicons were chosen because of the levels of expression of CEA observed in replicon infected cells. We extended these studies to another gene relevant to breast cancer, the HER2/neu oncogene. We constructed a replicon which encodes the extracellular domain of this oncogene. Expression of the HER2/neu protein was confirmed by immunoprecipitation with antibodies specific for the extracellular domain of HER2/neu.

We have also developed suitable methodologies for large scale production of the replicons encoding the different oncogenes. Included in this work was the development of methodologies to remove both the contaminating vaccinia virus (VVP1) that is used to generate the encapsidated replicons. Safety testing in the transgenic animals demonstrated that no recombinant vaccinia virus was present. In addition, we have also confirmed that no poliovirus exits in these preparations which might have been generated as a result of recombination with VVP1.

Analysis of the immunogenicity of encapsidated replicons encoding CEA or HER2/neu.

Experiments were undertaken to analyze the immunogenicity of replicons encoding CEA or HER2/neu. For these studies, we have made use of a mouse which is transgenic for the human receptor for poliovirus (13). This mouse is susceptible to poliovirus when given via intramuscular or intraperitoneal routes. Previous studies from this laboratory have demonstrated that the mouse can be used for analysis of the immunogenicity of encapsidated replicons (11).

Our first series of experiments used groups of 10 mice each. We immunized mice with encapsidated replicons which encode the C-fragment of tetanus toxin as a control. Mice were also immunized with replicons which encode the signal minus version of CEA, which was found to be our optimal expressing replicon from the *in vitro* experiments. We monitored the production of anti-CEA antibodies using a solid phase immunoassay. All mice immunized with the replicon encoding CEA generated an anti-CEA antibody response. The levels of the anti-CEA antibody response were variable, though, with a range of 250 ng of I^{125}-CEA bound per ml of sera to 17,000 ng of I^{125}-CEA bound per ml of sera. Values of approximately 15 ng per ml were found to be background. The variability of the immune response to CEA expressed from a replicon has been noted for other proteins expressed from replicons using these transgenic mice (11).

In a second series of experiments, we analyzed the immunogenicity of encapsidated replicons encoding HER2/neu. Mice were given 10^7 infectious units of encapsidated replicons encoding HER2/neu intramuscularly. Following three immunizations, the sera of the mice were tested for antibodies reacting to HER2/neu. For this analysis, we utilized cell lysates from a cell line known to express high levels of HER2/neu (SKOV.3). The extracts were subjected to SDS-PAGE followed by transfer nitrocellulose membranes. Using the sera from each mouse diluted 1 to 100, we performed a Western blot analysis.

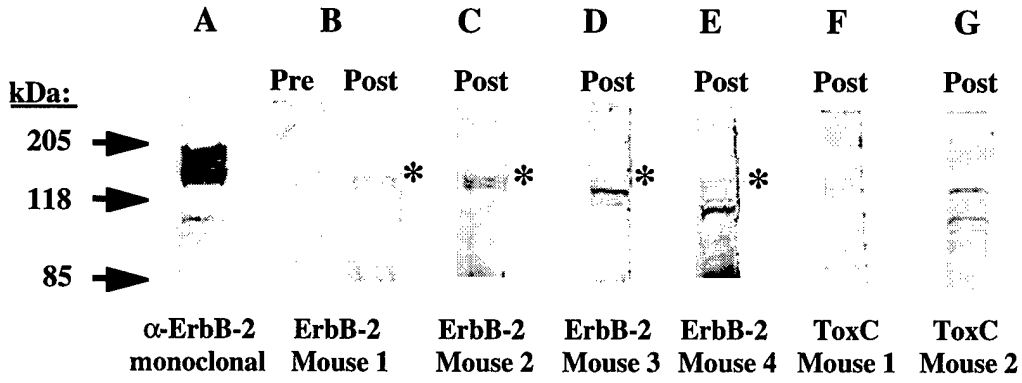

Figure 1. HER2-neu Replicons are Immunogenic in Mice. Mice were immunized a total of three times with replicons encoding HER2-neu or tetanus toxoid C-fragment (ToxC) at three week intervals. After the third immunization, sera were analyzed for HER2-neu-specific antibodies by using a Westen blot assay. A cell lysate from SKOV-3 cells, an ovarian carcinoma cell line that overexpresses human HER2-neu2, was used as the source of antigen on the blot. A monoclonal antibody specific for ErbB-2 was used as a positive control to detect the immobilized HER2-neu from the SKOV-3 cell lysate (Panel A). Sera from mice pre-immunzation did not detect any proteins (Panel B, Pre). Sera collected from four mice post-immunization with ErbB-2 replicons detected a protein consistent with that from the blot probed with the ErbB-2 monoclonal (Panels B-E). This protein was not detected on blots probed with post-immunization sera from mice given ToxC replicons, although other background proteins with a slightly faster mobility were detected at low levels (Panels F and G).

As can be seen, the immunized mice all responded with antibodies which reacted to the HER2/neu blotted onto the nitrocellulose membrane. Similar to what we have found in mice immunized with replicons encoding CEA, a variable level of anti-HER2/neu antibodies was observed among the mice. The results of these studies, though, clearly demonstrate that the replicons encoding HER2/neu were immunogenic when given via intramuscular routes to the transgenic mice.

Tumor Challenge of Immunized Mice.

During the last year's funding, we have demonstrated that inoculation of 2.5 x 10^5 MC38-CEA cells in the flank of the human poliovirus receptor transgenic mice by subcutaneous injection produced a measurable tumor outgrowth in greater than 90% of the mice within 4 weeks. These animals go on to die as a result of tumor burden. To determine if immunization with encapsidated replicons encoding CEA would result in anti-tumor immunity, we immunized 10 mice with 10^7 infectious units of the replicon encoding the signal minus version of CEA (CEA-Sig-). As a control, 10 animals were immunized with the replicon encoding the C-fragment of tetanus toxin. The results of the these challenge studies were very promising. None of the animals immunized with the replicon encoding CEA developed tumors, whereas all of the animals immunized with the control replicon (encoding C-fragment of tetanus toxin) went on to develop tumors and eventually die.

Replicon used for Immunization	Mice with Tumors/ Total Immunized
Not Immunized	8/10
Tetanus Toxoid C-fragment	10/10
Sig$^-$ CEA	0/10

Table 1. Tumor Challenge Studies in Mice Immunized with Replicons. Groups of ten mice were immunized with replicons encoding either the tetanus toxoid C-fragment or Sig-CEA. A third set was not immunized. The groups of mice were given booster immunizations of replicons three times, at three week intervals. After the last booster immunization, the mice were challenged with a tumor cell line (MC38-CEA) that expresses human CEA. The mice then were monitored for tumor development. The mice that developed tumors ultimately died; all of the mice immunized with the Sig-CEA replicon survived the challenge study.

Enhancement of the immunogenicity of encapsidated replicons encoding CEA.

In parallel experiments, we developed replicons which might be of use to enhance the immunogenicity of those replicons encoding CEA. Our strategy has been to develop replicons which encode biologically active immune modulators (cytokines) that would have utility as a biological adjuvant. Our initial studies have focused on two of the most commonly used cytokines for this purpose: GM-CSF and IL-2. We constructed replicons which encode the murine genes for GM-CSF and IL-2. Infection of cells with these replicons results in the production GM-CSF or IL-2 as measured by a commercially available ELISA (Quantikine Kit).

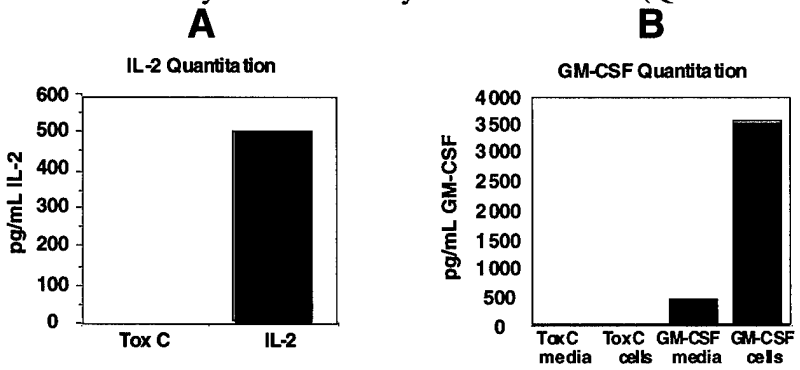

Figure 2. Expression of IL-2 and GM-CSF from replicons. $5x10^5$ Hela H1 cells were infected with replicons encoding either murine IL-2 or murine GM-CSF at a multiplicity of infection of 10 infectious units per cell. Cell lysates and/or media were collected at 6 hours post-infection and analyzed for murine IL-2 (Panel A) or murine GM-CSF (Panel B) by using a commercially available ELISA kit. The graphs show concentrations (pg/mL) of the cytokines detected in cell lysates (IL-2) or both cell lysates and culture supernatants (GM-CSF).

We determined that infection of cells with replicons encoding IL-2 or GM-CSF resulted in the production of 6,000 pg of IL-2 per 10^6 cells or 4,000 pg of GM-CSF per 10^6 cells, respectively. We have also determined that the cytokines produced are biologically active.

In a preliminary experiment, we immunized transgenic mice with soluble tetanus toxoid and the replicon encoding GM-CSF (10^8 infectious units per dose); as a control, mice were given only tetanus toxoid. A total of 2 immunizations were given (i.e. prime and boost). Sera from the mice were collected and the end point titer of antibodies to tetanus toxoid was determined. **Interestingly, we found that, on average, the mice co-immunized with replicons encoding GM-CSF had 3-4 fold higher titers two weeks after the last immunization. Follow-up studies of these mice revealed that the increase in anti-tetanus toxoid titer was maintained for approximately six weeks after the booster immunization.**

Further characterization of immune responses of replicons (last year of funding).

During the last year of funding, we concentrated our efforts into two areas. In the first, we further characterized the immune response generated by administration of replicons encoding foreign proteins via the intramuscular route. We found that co-administration of replicons encoding foreign proteins with replicons encoding cytokines did not enhance the immune response to the foreign protein. This was in contrast to our previous studies were we found that co-administration of replicon with a soluble protein (tetanus toxiod) resulted in an enhanced immune response to tetanus toxoid. We believe that this result was due to the fact that the replicons express proteins only for a limited time *in vivo*. Studies using a replicon encoding luciferase have found that the expression of luciferase can be detected for approximately 24-48 hours following *in vivo* administration. Thus, the reason why replicons encoding cytokines co-administered with replicons encoding foreign protein do not enhance the immune response was probably due to timing; that is, with both the cytokine and target protein produced at the same time, we probably were unable to optimally stimulate the immune response. Experiments are ongoing to further resolve this issue. We also began to dissect out features of the immune response which are stimulated by administration of replicons. We performed these experiments because we had seen variable levels of antibody produced to CEA or HER2/neu upon repeat experiments. We found that three intramuscular immunizations with replicons encoding foreign protein reproducibly stimulated an antibody response that was mainly IgG 2a. Isolation of spleen cells followed by re-stimulation with antigen revealed the production of IFN-γ without production of IL4. Thus, administration of replicons induced a predominantly TH1 response. We found

that animals immunized with the replicons followed by booster immunization with soluble protein antigen resulted in an enhanced antibody response to the target antigen. Our working hypothesis then is that replicon administration stimulates predominantly a CD4+ T cell help. We speculate that this CD4+ T cell help can be used for stimulation of a humoral as well as a cell mediated immune response depending upon the second antigen stimulus. Thus, our future studies that will be conducted out of the scope of this grant will utilize our replicons encoding CEA in combination with recombinant vaccinia virus which encodes CEA. We speculate that immunization with replicons encoding CEA will stimulate predominantly CD4+ T cell help which can be driven to produce a significant CTL response using vaccinia virus encoding CEA.

In a second line of experiments, we have further developed the tumor model required for future testing of our replicons. To do this, we have obtained the cDNA for the receptor for poliovirus. We have generated tumor mouse cell lines which express this receptor and thus become susceptible to poliovirus infection. These tumor mouse cell lines are also susceptible to replicon infection since replicons utilize the same receptor as poliovirus. We are now testing the capacity of these tumor cell lines to form tumors in the transgenic mice (we anticipate no problem with this study). Using this newly modified system, we will be able to fully characterize the effect that immunization with replicons has in the generation of an anti-tumor response.

CONCLUSIONS

The results carried our during this study have clearly established the potential for use of replicons encoding tumor antigens as a means to stimulate antitumor immune responses. The studies which we have conducted during the funding period have resulted in the development of replicons encoding two different tumor antigens (CEA and HER2/neu). This research, in combination with ongoing research in the laboratory, has established the key elements of the immune response generated by replicon vaccination following intramuscular immunization. In some instances, this immune response results in the production of antibodies as well as a cell mediated immune response which can lead to anti-tumor immunity. In most cases, though, we have found that the administration of the replicons results in the stimulation of a TH1 type response which can be boosted by administration of soluble antigen or, as we will determine in future experiments, the use of alternative vectors which can present antigens for stimulation of cytotoxic T lymphocytes such as recombinant vaccinia viruses. We plan to continue our studies using CEA and HER2/neu as model tumor antigens to further develop the vaccine strategies using replicons that we hope will ultimately be formulated into a vaccine strategy for micrometastases.

REFERENCES

1. Cancer Vaccines Get a Shot in the Arm (Research News). Science. 262:841-843.

2. Ansardi, D. C., Z. Moldoveanu, D. C. Porter, D. E. Walker, R. M. Conry, A. F. LoBuglio, S. McPherson, and C. D. Morrow. 1994b. Characterization of poliovirus replicons encoding carcinoembryonic antigens. Cancer Res. 54:6359-6363.

3. Berd, D., H. Maquire, and M. Mastrangelo. 1986. Induction of cell mediated immunity to autologus melanoma cells and regression of metastases after treatment with melenin cell vaccine. Cancer Res. 46:841-843.

4. Dranoff, G., E. Jaffee, and A. Lazenby. 1994. Vaccination with irradiated tumor cells engineered to secretemurine GM-CSF stimulates potent, specific and long lasting anti-tumor immunity. Proc. Natl. Acad. Sci. (USA). 90:3539-3543.

5. Gransbacher, B., R. Bannerji, and D. B. 1990. Retroviral vector mediated gamma interferon gene transfer into tumor cells generates potent and long lasting anti-tumor immunity. Cancer Res. 50:7820-7825.

6. Hanna, M., L. Peters, and H. Hoover. 1991. Immunotherapy by active specific immunization: Basic principles and preclinical studies. In Biologic Therapy of Cancer by DeVita, Hellman and Rosenberg (eds), J.P. Lippincott Co., Philadelphia,. 651-670.

7. Hellstrom, K. E., and I. Hellstrom. 1992. Possibilities for active immunotherapy of human cancer. Cancer Invest. 10:285-293.

8. Koch, F., and G. Koch. 1985. The molecular biology of poliovirus. Springer-Verlag Vienna.

9. McCune, C., D. Schapira, and E. Henshaw. 1981. Specific immunotherapy of advanced renal carcinoma. Cancer. 47:1984-1987.

10. Mitchell, M., W. Hard, and R. Kempf. 1990. Active specific immunotherapy for melanoma. J. Clin. Onc. 8:856-869.

11. Moldoveanu, Z., D. C. Porter, A. Lu, S. McPherson, and C. D. Morrow. 1995. Immune responses induced by administration of encapsidated poliovirus replicons which express HIV-1 gag and envelope proteins. Vaccine. 13:1013-1022.

12. Pardoll, D. 1992. New strategies for active immunotherapy with genetically engineered tumor cells. Current Opinion in Immunol. 4:619-623.

13. Ren, R., and V. R. Racaniello. 1992. Poliovirus spreads from muscle to the central nervous system by neural pathways. J. Infect. Dis. 166:747-752.

Dr. Casey D. Morrow
DAMD17-96-J-4403
Bibliography

Manuscripts

1. Ansardi, DC, Moldoveanu, Z, Porter, DC, Walker, DE, Conry, RM, LoBuglio, AF, McPherson, S, Morrow, CD. Characterization of poliovirus replicons encoding carcinoembryonic antigen. Cancer Research. 1994. 54:6359-64.

2. Morrow, CD, Ansardi, DC, Porter, DC. 1996. Methods for the use of poliovirus vectors for gene therapy. The Humana Press Inc., Methods in Molecular Biology. Chapter 14:1-17.

3. Basak, S., S. McPherson, S.F. Kang, J. Collawn, and C.D. Morrow. 1998. Construction and characterization of encapsidated poliovirus replicons which express biologically active murine interleukin-2 (mIL-2). J. Interferon and Cytokine Res. 18:305-313.

4. Morrow, C.D., M.J. Novak, D. C. Ansardi, D.C. Porter, Z. Moldoveanu. 1998. Recombinant viruses vectors for mucosal immunity. In: *Current Topics in Microbiology and Immunology*, J-P Kraehenbuhl, M. Neutra (eds). Springer-Verlag, Berlin-Heidelberg.

Meeting/Abstracts

1. SELF REPLICATING RNA (REPLICON)-BASED IMMUNOTHERAPY FOR BREAST CANCER. Dr. David C. Ansardi, Lucretia Sumerel, Robert M. Conry, and Dr. Casey D. Morrow. Department of Defense. US Army Medical Research and Materiel Command, Breast Cancer Research Program: An Era of Hope, Washington, D.C., October 31-November 4, 1998

Personnel Receiving Pay from DAMD 17-94-4403

Casey D. Morrow, Ph.D.
Albert F. LoBuglio, M.D.
Robert M.Conry, M.D.
David C. Ansardi, Ph.D.
Donna C. Porter, Ph.D.
Sang-Moo Kang, Ph.D.
Jun Wang
Margaret K. Whitfield
Susan E. Moore