AD NUMBER
ADB254454

NEW LIMITATION CHANGE

TO
Approved for public release, distribution unlimited

FROM
Distribution authorized to U.S. Gov’t. agencies only; Proprietary Info.; Aug 99. Other requests shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott St, Fort Detrick, MD 21702-5012.

AUTHORITY
USAMRMC ltr, 23 Aug 2001
Award Number: DAMD17-99-1-9039

TITLE: Antisense Down-Regulation of Metallothionein: A Therapeutic Approach for Prostate Cancer

PRINCIPAL INVESTIGATOR: Asim B. Abdel-Mageed, Ph.D.

CONTRACTING ORGANIZATION: Tulane University School of Medicine
New Orleans, Louisiana 70112-2699

REPORT DATE: August 1999

TYPE OF REPORT: Final

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

DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, Aug 99). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
NOTICE

USING GOVERNMENT DRAWINGS, SPECIFICATIONS, OR OTHER DATA INCLUDED IN THIS DOCUMENT FOR ANY PURPOSE OTHER THAN GOVERNMENT PROCUREMENT DOES NOT IN ANY WAY OBLIGATE THE U.S. GOVERNMENT. THE FACT THAT THE GOVERNMENT FORMULATED OR SUPPLIED THE DRAWINGS, SPECIFICATIONS, OR OTHER DATA DOES NOT LICENSE THE HOLDER OR ANY OTHER PERSON OR CORPORATION; OR CONVEY ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE, OR SELL ANY PATENTED INVENTION THAT MAY RELATE TO THEM.

LIMITED RIGHTS LEGEND

Award Number: DAMD17-99-1-9039
Organization: Tulane University Medical Center

Those portions of the technical data contained in this report marked as limited rights data shall not, without the written permission of the above contractor, be (a) released or disclosed outside the government, (b) used by the Government for manufacture or, in the case of computer software documentation, for preparing the same or similar computer software, or (c) used by a party other than the Government, except that the Government may release or disclose technical data to persons outside the Government, or permit the use of technical data by such persons, if (i) such release, disclosure, or use is necessary for emergency repair or overhaul or (ii) is a release or disclosure of technical data (other than detailed manufacturing or process data) to, or use of such data by, a foreign government that is in the interest of the Government and is required for evaluational or informational purposes, provided in either case that such release, disclosure or use is made subject to a prohibition that the person to whom the data is released or disclosed may not further use, release or disclose such data, and the contractor or subcontractor or subcontractor asserting the restriction is notified of such release, disclosure or use. This legend, together with the indications of the portions of this data which are subject to such limitations, shall be included on any reproduction hereof which includes any part of the portions subject to such limitations.

THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.

M. F. O'Brien
5/25/00
Antisense Down-Regulation of Metallothionein: A Therapeutic Approach for Prostate Cancer

Asim B. Abdel-Mageed, Ph.D.

Tulane University School of Medicine
New Orleans, Louisiana 70112-2699

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

Despite significant research in the field of prostate cancer (PCa), the disease remains elusive in terms of its etiology, pathophysiology and clinical management. Identification of new genotypic and phenotypic markers underlying initiation, promotion and endocrine therapy failure would be of paramount significance. We have shown that metallothionein (MT) can induce mitogenic response in tumor cells and that its inhibition can lead to growth arrest and induction of apoptotic cell death. The objective of this training grant was to establish an antisense MT adenovirus construct and test its efficacy on growth inhibition and induction of apoptosis in vitro and in vivo. Due to experimental difficulties and the short time-frame specified for this award (6 months) we were able only to generate the antisense MT adenoviral construct. The experiments testing the efficacy of this novel construct are ongoing. The significance of this construct lies on the fact that MT is a mitogenic protein and its abundance, as shown in many human primary tumors, is associated with development of drug resistance and poor prognostic outcome. Thus, it is anticipated that this novel antisense construct will have a significant impact on growth inhibition and enhancement of efficacy of chemotherapeutic agents.
Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Where copyrighted material is quoted, permission has been obtained to use such material.

Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

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.

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.

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

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

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

[Signature]

PI - Signature  Date
(3) **Table of Contents**

<table>
<thead>
<tr>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Front Page</td>
</tr>
<tr>
<td>2. Report Documentation Page (SF 298)</td>
</tr>
<tr>
<td>3. Foreword</td>
</tr>
<tr>
<td>4. Table of Contents</td>
</tr>
<tr>
<td>5. Introduction</td>
</tr>
<tr>
<td>6. Body</td>
</tr>
<tr>
<td>7. Key Research Accomplishments</td>
</tr>
<tr>
<td>8. Reportable Outcome</td>
</tr>
<tr>
<td>9. Conclusions</td>
</tr>
<tr>
<td>10. References</td>
</tr>
<tr>
<td>11. Appendices</td>
</tr>
</tbody>
</table>

4
Introduction:
Prostate cancer (PCa) still poses question regarding its etiology, pathology, pathogenesis and clinical management. It has been estimated that there may be quarter of a million cases of PC worldwide (1) and the incidence of PC is projected to increase significantly (2). While earlier reports estimated that PC account for 35,000 deaths annually in Europe (3) and 38,000 in the USA (4), recent reports demonstrated that the difference between the incidence rate and the death rate is striking; in 1996 there were 317,000 newly diagnosed PC cases and 41,400 deaths in the USA compared to the 1995 figures of 244,000 and 40,400, respectively (5). However, it should be noted that there have been no major changes in mortality and no major advances in treatment parallel with the current rise in diagnosis since the introduction of androgen down-regulation regimen. Recent figures on prostate cancer suggest that the incidence vary considerably by race and age (6, 7). It has been shown that the mortality rates for African-American men were 2.2 times higher than those for white men. In more details, addition, the mortality rate among African-Americans has increased 68% between 1960-62 and 1990-92, and in recent years, have increased approximately 4% annually (8).

Despite significant research in the field of PCa, the disease remains elusive in terms of its pathogenesis and clinical management. Identification of new genotypic and phenotypic markers and characterization of molecular mechanisms underlying promotion of PCa to metastatic phenotypes and endocrine therapy failure would be of paramount significance. Several lines of evidence demonstrate a relationship between metallothionein (MT) expression in various types of human primary tumors and disease progression, metastasis, and poor prognostic outcome. MTs are a set of ubiquitous, low molecular weight (6-7kD) aromatic amino acid-free proteins that have 20 reduced cysteine residues which can chelate seven bivalent heavy metal ions through mercaptide bonds (9). In humans, MTs are encoded by a family of genes located at 16q13 (10,11) that contains 10 functional and 7 nonfunctional isoforms (12,13). The gene sequence and expression of all the functional human MT genes have been identified and well characterized: MT-IIA (14), MT-3 (15), MT-4 (16), MT-IA (17), MT-IB (18), MT-IE, MT-IF and MT-IG (19), and MT-IH and MT-IX (20).

MT overexpression has been shown to be associated with disease progression, metastasis, and poor prognostic outcome in a number of human primary tumors, including malignant melanomas (21), ovarian tumors (22), testicular germ cell tumors (23), bladder transitional cell carcinomas (24), and bladder cancer and renal cell carcinoma (25). However, the functional role of MT in tumor cell growth is poorly understood. To answer this question, we have recently demonstrated in breast carcinoma MCF-7 cells that MT overexpression enhances cellular proliferation and c-myc gene expression and suppresses c-fos and p53 transcripts, whereas antisense down-regulation of MT induces growth arrest and apoptotic cell death (26). We also showed that the activation of transcription factor NF-kappa B and its interaction with MT may be required for the MT-mediated mitogenic response (27).

In our laboratory, we demonstrated the isoform-specific expression of MT in PCa and further examined the extent to which they modulate disease progression and metastasis. Specifically, our preliminary data using RT-PCR have shown enhanced MT-IIA, MT-IE, and MT-IF gene expression in human PCa biopsy samples when compared to tissues from benign prostatic hyperplasia (BPH), suggesting that at least the aberrant expression of specific MT isoforms(s) may be associated with growth promotion and metastatic disease. This observation was confirmed by IHC localization showing enhanced MT protein expression in cancer cells, basement membrane and lumen secretory products in biopsy specimens from PCa patients when compared to BPH specimens. Our preliminary data also indicated that MT-IIA overexpression alone has enhanced cell multiplication, collagenase (MMP-2) and MSA in a panel of PCa cell lines, LNCaP, PC-3, and DU-
These preliminary data have led us to hypothesize that MT plays a pivotal role in the promotion of neoplastic cell growth and in the progression of PCa to metastatic disease. Therefore, in this proposal we seek to ascertain whether the down-regulation of MT gene by antisense adenoviral construct will be able to inhibit growth and metastasis of prostate cancer cells.

(II) Objectives: To further test this hypothesis we proposed the following specific aims: (a) to construct an MT antisense adenoviral vector for achieving high transfection efficiency in human prostate cancer cells, LNCaP, DU-145 and PC-3; (b) to determine in vitro growth inhibitory effects of the MT antisense adenoviral construct; (c) to determine the MT antisense induced apoptosis by quantitative DNA fragmentation and TUNNEL methodology; (d) to monitor tumor growth delay and modulation of metastasis in athymic nude mice after transplantation of transfected cells.

(6) Body:

a. Optimization of transfection efficiency of prostate cancer cells with adenoviral vectors.

Adv5-β-galactosidase reporter construct was generously provided by Dr. David T. Curiel, University of Alabama at Birmingham, AL. Subconfluent cultures of prostate cancer cell lines, DU-145, PC-3 and LNCaP, were exposed to various concentrations of the reporter construct (10-100 MOI) and transfection efficiency was determined 24 hr post-transduction by measuring β-galactosidase activity in situ. Briefly, the cells were washed in phosphate buffered saline (PBS) and fixed (formaldehyde/formaldehyde solution) at room temperature for 5 min. Cells were then stained (potassium ferricyanide/ferrocyanide, MgCl₂ and X-gal) 2 hr to overnight at 37°C, washed in PBS and observed on an inverted microscope. Efficiency of transfection was measured by scoring dark blue cells positive. The results demonstrated that 100 MOI is optimum for achieving 95-100% transfection efficiency in all cell lines. The results are depicted in Figure 1.

Figure 1: Subconfluent cultures of PC-3 cells were transfected with Adv5-β-gal vector (100 MOI) for 24 hr. Cells were then fixed and stained for β-galactosidase activity in situ as shown above. The results demonstrated that adenoviral constructs can efficiently express a transgene (95-100%) in prostate epithelial cells.
b. Construction of an MT Antisense Adenoviral Vector.

Plasmids for adenovirus vector construction for transgene insertion in the E1 region of Ad5 vectors was purchased from Mirobix Biosystems Inc, Ontario, Canada. The kit is composed of the shuttle vector, pMH5, the circular replication-defective Ad genome plasmid pJM17A and the low-passage packaging cell line, 293 cells (Figure 2 below).

![Diagram of adenovirus construction system](image)

**Figure 2:** Adenovirus construction system: shuttle plasmid pMH5 (a) and replication-defective circular viral vector pJM17 (b); intracellular recombination (c).

5' EcoRI /3' XhoI' fragment encompassing the cDNA of mouse MT-I was initially digested with the restriction enzymes and subsequently removed from agarose gel by electroelution using Bio-Rad electrophoreser (Figure 3). To subclone the digested fragment in reverse (or antisense) or the sense orientation (control) in the E1 region of shuttle plasmid, 5 micrograms of the pMH5 plasmid (purchased from Microbix Biosystems, Inc, Ontario, Canada, see map below) was initially digested with EcoRI and subsequently dephosphorylated using calf intestinal phosphatase (CIP) enzyme to prevent re-ligation. Ligation was carried out in accord with manufacture's instructions (Promega) using 1X buffer containing dATP and 3 U of T4 DNA ligase in 50 μl final volume at 16°C overnight. The reaction was stopped by heating at 75°C for 10 min. The overhangs were then filled using Klenow fragment (1 U) (Promega) in presence of dNTPs at 30°C for 10 minutes and reaction was stopped by heating at 75°C for 10 min. Blunt end ligation was carried out as shown above in presence of 6 U of T4 DNA ligase overnight. Reaction was stopped and samples were stored at -20°C until used.

**Figure 3:**

<table>
<thead>
<tr>
<th>Lane</th>
<th>Marker</th>
<th>Digest Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>λ-Hind-III</td>
<td>mMT-I digest</td>
</tr>
<tr>
<td>2</td>
<td>mMT-I</td>
<td>5'EcoRI/XhoI' digest</td>
</tr>
</tbody>
</table>
b. Transformation.

Competent *E. coli* DH₅₅ was transformed with plasmids in accord with manufacturer’s instructions. Briefly, bacterial cells were preincubated in ice with recombinant or control plasmids for 30 minutes prior to temperature shock at 42°C for 1.5 min. Bacterial cells were then grown in 1 ml LB with (shaking at 200 cpm) in antibiotic-free medium for 1 hr at 37°C. Cultures were then transferred into flasks containing 100 ml LB supplemented with ampicillin (50 μg/ml) and subsequently grown for an additional 24 hr. Bacterial cell growth was detected spectrophotometrically. Samples of culture media were further cultured in LB agar plates containing ampicillin at 50 μg/ml. Individual colonies (20) picked and further grown in LB agar plates.

c. Isolation of plasmid DNA.

Mini preps were used for individual clones by culturing bacteria in 10 ml LB media containing ampicillin overnight. DNA was isolated using standard methods. Briefly, Harvested overnight cultures were pelleted, washed in TE buffer, resuspended in GTE buffer containing lysozyme and incubated at 30°C for 20 min. Cell were lysed in presence of SDS and NaOH and plasmid DNA was isolated by centrifugation in presence of 3M K⁺ 5M OAc, pH 5.6. Ribonucleic acids were then removed using RNase A at 37°C for 30 min. DNA was extracted twice using phenol/chloroform/isoamyl alcohol (24:24:1) and precipitated with 1 volume of isopropanol. The pellets were washed once in 70% ethanol and stored at -20°C for further analysis.

d. Analysis of recombinant plasmids by restriction mapping.

To determine whether our DNA inserts (sense and antisense) were successfully cloned in the shuttle plasmid pMH5, we analyzed the DNA plasmids using 0.7% agarose gel containing ethedium bromide.

e. Generation of recombinant shuttle plasmids. Our results showed that among the twenty clones picked up for analysis of DNA inserts, none had shown a band or a band shift in the gel, as opposed to controls. We then decided to repeat the subcloning, transformation and analysis steps shown above without any successful attempt to subclone the mMT-I fragment. Our results have indicated that the bacteria were successfully cloned with the control plasmids, but not with our recombinant shuttle plasmids.

Even though we scrutinized all of our steps to subclone the DNA inserts we were unable to detect why this system have failed (see results below). We concluded that, the shuttle plasmid we purchased may have defective multiple cloning site at the EcoR1 site.
f. Use of pAdantage System for construction of MT antisense vector.

Our next approach was to use an alternative system for cloning hMT-II inserts in both orientation. A novel cloning method for recombinant adenovirus construction in Escherichia coli has been developed by Genzyme Co, Framingham, MA (28). The pAdantage system was a generous gift by Dr. D. W. Souza Genzyme Corporation. The system is composed of the shuttle plasmid pSV-ICEU I (containing the cytomegalovirus promoter (CMV), multiple cloning site (MCS) and polyadenylation site)(Figure 3a) and the viral vector pAdantage (Figure 3b). Because of close homology between human metallothionein and the use of human prostate cancer cells, we decided to use human MT-IIa cDNA (pBacNEO-sMT-IIa) to generate the antisense adenovirus constructs (Figure 3c).

Figure 3: pAdantage system: (a) the shuttle plasmid pSV-ICEU I; (b) pAdantage viral vector; and (c) pBacNEOSMT-IIa.

g. Isolation of hMT-IIa from pBacNEO-sMT-IIa plasmid.

Twenty microgram of pBacNEO-sMT-IIa plasmid was initially digested with 3' XhoI- KpnI 5' for 2 hr at 37°C. The products were then fractionated into a 0.7% agarose gel containing ethedium bromide. A DNA fragment (approx 300 bp) encompassing hMT-IIA was then isolated using electroelution technique in accord with the manufacturer instructions (Figure 4). Briefly, gel slices containing the DNA were placed in an apparatus equipped with tubes capped with special membranes and DNA was collected in the membranes by electroelution and subsequently precipitated using standard techniques. The cDNA was then stored at -20°C until used.
and extension at 59°C for 15 sec. The products were then fractionated into a 0.7% agarose gel containing ethedium bromide. A DNA fragment (approx 300 bp) encompassing hMT-IIA was then isolated using electroelution technique in accord with the manufacturer instructions (Figure 4). Briefly, gel slices containing the DNA were placed in an apparatus equipped with tubes capped with special membranes and DNA was collected in the membranes by electroelution and subsequently precipitated using standard techniques. The cDNA was then stored at -20°C until used.

![Agarose gel electrophoresis](image)

Figure 4: (a) Agarose gel electrophoresis (0.7%) showing 3'XhoI- KpnI5' hMT-IIa fragment (approximately 320 bp); lane 1 3'XhoI- KpnI5 double digest, lane 2 DNA marker ; (b) PCR amplification of hMT-IIa using pBAcNEOs-MT-IIa as a template, lane 1, hMT-IIa; lane 2, pBAcNEOs-MT-IIa plasmid

**h. Cloning of hMT-IIa in the shuttle plasmid pSV-ICEU I.**

*Preparation of the shuttle plasmid:*

The shuttle plasmid pSV-ICEU I was initially digested with XhoI, dephosphorylated with calf intestine phosphatase (CIP) to prevent re-ligation, and precipitated with phenol/chloroform in accord with standard techniques and stored -20°C until used.

*Ligation:*

For preparation of the antisense construct, the 3'XhoI- KpnI5' fragment encompassing the human MT-IIa was initially ligated to the shuttle plasmid pSV-ICEU I using 1X ligation buffer, 3 U of T4 DNA ligase, 0.25 µg of shuttle plasmid with three-fold molar excess of the DNA insert at 15°C overnight. The reaction was stopped at 75°C for 10 min. The overhangs were then filled with Klenow fragment (1 U) (Promega) in presence of dNTPs at 30°C for 10 minutes and reaction was stopped by heating at 75°C for 10 min. Blunt end ligation was carried out as shown above in presence of 400 U of T4 DNA ligase (New England Biotechnology) overnight. The reaction was stopped and samples were stored at -20°C. For the preparation of the control sense plasmid, both XhoI-digested pSV-ICEU I and 3'XhoI- KpnI5 fragment were initially filled using Klenow fragment as shown above to generate blunt ends and ligation was carried out using high concentration of T4 DNA ligase (New England BioLabs) at 15°C overnight.
**Transformation and plasmid Isolation:**

Competent *E. coli* DH<sub>5</sub>α was transformed with plasmids in accord with recommended procedure as described above. Mini preps were used for isolation of individual clones in accord with standard protocol as shown above.

**Analysis of ligation:**

The fidelity of DNA insertion was analyzed using 0.7% agarose gel containing ethedium bromide. The results demonstrated that hMT-IIa was efficiently subcloned in the shuttle vector (Figure 5). Initially, all colonies screened for the control vector have shown no DNA insert and we had repeated the whole cloning techniques before we were successful on isolating a positive clone.

![Figure 5: Gel electrophoresis demonstrating a size shift in the recombinant shuttle vector as opposed to control plasmid. M, DNA marker; lane 1-5 MT-IIa antisense recombinant clones; lanes 7-9 control MT-IIa (sense) recombinant clones; lanes 10-12, shuttle plasmid](image)

**Analysis of orientation:**

The orientation of the DNA insert was performed using restriction mapping of the recombinant plasmids followed by analysis using 0.7% agarose gel electrophoresis. The results demonstrated that the transgene is in the antisense orientation.

![Figure 6: Digestion of the recombinant plasmids with ScaI and BamHI. B. It is anticipated that three (lanes 1-3) fragments will be generated following the double digestion in the positive clones. Lane 3 represents a positive clone for the MT antisense clone. M, DNA marker.](image)
Digestion of recombinant plasmid with I-Ceu I:

A fragment encompassing CMV promoter-antisense MT-IIa cDNA and poly A regions was digested from the recombinant shuttle plasmid pSV-ICEU I using I-Ceu I (New England Biolabs). The DNA insert was then isolated using agarose gel electrophoresis and electroelution as described above. After precipitation, the pellet was stored at -20°C.

J. Subcloning of the antisense MT-IIa from pSV-ICEU I into the I-Ceu I site of the pAdvantage viral vector.

Preparation of pAd
tage

Five micrograms of pAd
ntage were digested with 30 U of I-Ceu I in a total volume of 500 μl of 1 X I-Ceu I buffer at 37°C for 3 hr. 10 units were added every hr to reach 30 U by the end of the 3 hr period. The plasmid was then dephosphorylated using CIP as shown above for an additional 20 min at room temperature and DNA was extracted using phenol/chloroform and precipitated with ethanol as shown above.

Subcloning of the I-Ceu I fragment into the I-Ceu I-site of the pAd
ntage:

The viral vector (0.25 μg) and three-fold molar excess of the I-Ceu I DNA insert were incubated at 16°C overnight in presence of 400 U of T4 DNA ligase and 1 X ligase buffer in a total volume of 50 μl. The fidelity of the DNA insert in the viral vector was verified by transformation, screening of putative colonies, and by agarose gel electrophoresis when compared to the viral vector alone as we showed above.

Figure 7: Agarose gel electrophoresis (0.7%, TAE Buffer) for analysis of MT antisense adenovirus construct. A representative clone (lanes 1) was analyzed for efficient ligation of I-Ceu I fragment into the I-Ceu I-site of the pAd
ntage and compared to the control plasmid (lane 2). A shift in band size in lane 1 as opposed to lane 2 indicates successful ligation.

k. 293 Cell Culture.

Low passage 293 cells, a human embryo kidney cells transformed by sheared Adenovirus type 5 DNA, were obtained from Microbix Biosystems Inc., (Ontario, Canada). They contain and express the early region (E1) of Ad 5 virus and can complement the E1 defective Adv mutants and vectors. The cells were grown in MEM supplemented with Earle’s salts, glutamine, 10% heat-inactivated FBS, penicillin and streptomycin and incubated at 37°C with 5% CO₂.
1. Viral Packaging in 293 cells.
   Preparation of the viral constructs (by first digesting the construct with SnABl to expose the inverted terminal repeats) for packaging in 293 cells is now underway and it is anticipated the viral plaques will become visible in about 7-10 days.

(7) Key Research Accomplishments:
   - Generation of novel recombinant antisense MT adenovirus vector.

(8) Reportable outcomes:
   - Construction of metallothionein MT adenovirus construct.
   - Applied for funding for a DOD grant in 1999 entitled “Antisense down-regulation of metallothionein: a combined therapeutic approach for prostate cancer.”
   - The data will be also used for funding application to the NIH, ACS and CaPCure.

(9) Conclusions.
   The aim of this training grant was to construct a metallothionein antisense adenovirus vector and test its efficacy on enhancing growth arrest and inducing apoptosis in cancer cells using in vitro and in vivo approaches. Like other drug resistance genes, metallothionein has been shown to play a role in protecting cancer cells against chemotherapeutic agents and radiotherapy—thus this vector can also be used to enhance sensitization of cancer cells to chemo/radiotherapy. Because this application was a training grant (no funds for research supplies or other personnel), and due to the unexpected research problems, I wasn’t able to accomplish all specific aims within the short-time frame specified for the grant (6 months). One recommendation, at least, is to include funding for research supplies and to extend the time-frame for these training grants to 1-2 years, so that the research will be completed.
References.


11. Appendices: N/A
MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to the technical reports listed at enclosure. Request the limited distribution statement for these reports be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management
Reports to be Downgraded to Unlimited Distribution

| ADB241560 | ADB253628 | ADB249654 | ADB263448 |
| ADB251657 | ADB257757 | ADB264967 | ADB245021 |
| ADB263525 | ADB264736 | ADB247697 | ADB264544 |
| ADB222448 | ADB255427 | ADB263453 | ADB254454 |
| ADB234468 | ADB264757 | ADB243646 |        |
| ADB249596 | ADB232924 | ADB263428 |        |
| ADB263270 | ADB232927 | ADB240500 |        |
| ADB231841 | ADB245382 | ADB253090 |        |
| ADB239007 | ADB258158 | ADB265236 |        |
| ADB263737 | ADB264506 | ADB264610 |        |
| ADB239263 | ADB243027 | ADB251613 |        |
| ADB251995 | ADB233334 | ADB237451 |        |
| ADB233106 | ADB242926 | ADB249671 |        |
| ADB262619 | ADB262637 | ADB262475 |        |
| ADB233111 | ADB251649 | ADB264579 |        |
| ADB240497 | ADB264549 | ADB244768 |        |
| ADB257618 | ADB248354 | ADB258553 |        |
| ADB240496 | ADB258768 | ADB244278 |        |
| ADB233747 | ADB247842 | ADB257305 |        |
| ADB240160 | ADB264611 | ADB245442 |        |
| ADB258646 | ADB244931 | ADB256780 |        |
| ADB264626 | ADB263444 | ADB264797 |        |