NEW LIMITATION CHANGE

TO
Approved for public release, distribution unlimited

FROM
Distribution authorized to U.S. Gov’t. agencies only; Proprietary Info.; Jul 96. Other requests shall be referred to Commander, Army Medical Research and Materiel Command, Attn: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012.

AUTHORITY
USMRMC ltr dtd 6 May 98
GRANT NUMBER DAMD17-94-J-4278

TITLE: Development of Ligand-Transformed Alpha-Fetoprotein for Use Against Breast Cancer in Humans

PRINCIPAL INVESTIGATOR: James A. Bennett, Ph.D.

CONTRACTING ORGANIZATION: Albany Medical College
Albany, New York 12208

REPORT DATE: July 1996

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, July 1996). Other requests for this document shall be referred to Commander, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RMI-S, Fort Detrick, Frederick, MD 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.
DISCLAIMER NOTICE

THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.
There is an isoform of alpha-fetoprotein (AFP) that stops breast cancer growth. Our goal is to establish conditions under which this active form can be produced and applied as a breast cancer therapeutic.

During the first two years of this grant, we have established these conditions and have shown anti-breast cancer activity with both natural and recombinant human AFP. We have shown that all of the activity resides in the third domain of the molecule and are in the process of producing in peptide form the active site of the molecule. The active form of the molecule produces a G1 block in the cell cycle. Thus far, estrogen-receptor-positive but not estrogen-receptor-negative tumors have been sensitive to AFP-induced oncostasis. An intermediate marker of activity is an increase in serum estrogen levels and perhaps FSH levels. There has been no evidence of host toxicity during therapeutic application of the active form of AFP.
Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US 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.

James A. Bennett 7-26-96

Signature          Date
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Front Cover</td>
<td>1</td>
</tr>
<tr>
<td>SF 298 Form</td>
<td>2</td>
</tr>
<tr>
<td>Foreword</td>
<td>3</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>4</td>
</tr>
<tr>
<td>Introduction</td>
<td>5</td>
</tr>
<tr>
<td>Body</td>
<td>8</td>
</tr>
<tr>
<td>Conclusions and Future Work</td>
<td>13</td>
</tr>
<tr>
<td>References</td>
<td>14</td>
</tr>
<tr>
<td>Bibliography of Publications</td>
<td>17</td>
</tr>
<tr>
<td>Personnel Receiving Pay</td>
<td>18</td>
</tr>
</tbody>
</table>
INTRODUCTION

Subject: We are studying the regulation of breast cancer growth by alpha-fetoprotein (AFP). AFP is a glycoprotein normally produced during gestation, initially by the fetal yolk sac and then by the fetal liver (1). It is a major protein constituent of the fetal plasma throughout gestation and has structural similarities to albumin (2). However, upon parturition, the gene for AFP is repressed, and its serum concentration diminishes to a negligible level. It is reexpressed during liver pathology such as hepatoma or cirrhosis. The restricted presence of this embryonic protein suggests a unique role for AFP in cell growth and differentiation, which are the hallmarks of embryonic life. Evidence for this role has been obtained in a variety of studies showing that AFP can regulate the growth and function of certain tissues such as liver (3), lymphocytes (4), placenta (5), ovaries (6), and uterus (7), and interact with certain ligands such as arachidonic acid (8), docosahexaenoic acid (8) and retinoic acid (8), all of which influence differentiation. Our own studies have shown that when either rodent or human AFP is incubated with a molar excess of estradiol, the protein undergoes a change in conformation (9). In this transformed state (tAFP), nanogram quantities of the material inhibit the growth of estrogen-stimulated tissues in vivo, including estrogen-stimulated breast cancers (10-13).

The physiological role of AFP, and especially tAFP, may be to act as a rudimentary servo mechanism that desensitizes endocrine tissues to the inappropriately high levels of steroid hormones that occur during gestation. This mechanism is fetoprotective, as the fetus develops in the presence of a large concentration of maternal and placental steroid hormones, and has receptors for these hormones, but it does not have the sophisticated control mechanisms of late fetal or adult life to regulate the production of and response to these hormones. A “side effect” of the proposed “servo mechanism” would occur when tAFP crossed the placenta into the maternal circulation where it would extinguish microscopic premalignant and/or cancerous foci in the breast that later on in life would be promoted to clinically detectable breast cancers. Such a “side effect” would explain the epidemiological data, which clearly show that the experience of full-term pregnancy decreases the lifetime risk of breast cancer (14).

Purpose: The purpose of our study is to produce large quantities of the active form of AFP and assess its effectiveness in the control of estrogen-stimulated growth of experimental human breast cancers.

The specific aims of our original grant proposal were:

1. Determine optimal reaction conditions between recombinant AFP and appropriate ligand to form tAFP. Then, maximize the antitumor activity of tAFP by manipulating its dose and schedule without introducing host toxicity in mice bearing human breast cancer xenografts.
2. Determine markers on tumors which predict tumor sensitivity to tAFP.
3. Determine intermediate markers in the host which indicate that tAFP is active in vivo.
4. Assess through histomorphometric studies the type of damage (lethal or non-lethal) done to the tumor by tAFP.

These aims are specifically designed so that, upon their completion, the tools will be available for clinical trial of tAFP for breast cancer.
Background: There are experiments of nature and laboratory experiments which point to AFP as a regulator of estrogen-stimulated growth of normal and malignant tissues. This has implications for AFP in the prevention and treatment of breast cancer, because almost all breast cancers start out as estrogen-receptor-positive and are stimulated in their growth by estrogen. By the time breast cancer is diagnosed, half of these breast cancers have further dedifferentiated to an estrogen-receptor-negative phenotype.

The evidence which supports the idea that AFP inhibits the response of tissues to estrogen is as follows. It is a well known fact that hepatomas secrete AFP (15). In fact, serum AFP levels are used as a marker of tumor burden in this disease. What is less well known is that amenorrhea is one of the first symptoms of hepatoma in premenopausal women and this symptom resolves following surgical removal of the tumor (16). Also, hyperestrogenemia and normal to elevated gonadotropins are present in hepatoma patients (17). Taken together, these data suggest that neither the uterus nor the hypothalamic-pituitary axis are responding to estrogen in hepatoma patients and, based on the remaining data to be discussed below, elevated AFP levels could bring about this result. Our own studies have shown that there is an isoform of AFP which upon exposure to estradiol, takes on a conformation that inhibits the estrogen-stimulated growth of normal mouse uterus (7). Soto et al. (18) have shown that AFP-containing serum from a hepatoma-bearing rat inhibits the estrogen-stimulated induction of progestin receptor. These same investigators have shown that an AFP-secreting tumor induces the regression of an estrogen-dependent tumor (19). An experiment of nature suggests that AFP is the factor in pregnancy which confers on parous women their significant reduction in risk of breast cancer. As shown in Table 1a, AFP is elevated in maternal serum during pregnancy. Furthermore, there are factors in pregnancy such as maternal race, weight, hypertension, consumption of alcohol, number of fetuses in utero, and neural tube defect in the fetus where maternal serum AFP (MSAFP) is substantially altered from normal pregnancy levels. In studying the literature, we have found the consistent and striking correlation that in those pregnancy conditions associated with an elevated level of MSAFP, there was a significant reduction in the lifetime risk to the mother of acquiring breast cancer. Conversely, in pregnancy conditions characterized by low MSAFP (alcohol), subsequent breast cancer risk was elevated (Table 1a). We carried out epidemiologic studies analyzing retrospective data that extend and confirm the correlation between MSAFP levels and breast cancer risk (Table 1b). Recently Ekbom et al. (33) have published an epidemiological study which suggests that, at least in the case of hypertension during pregnancy, the reduction of breast cancer risk is also passed on to the fetus. He is in agreement with our speculation that it is AFP in the fetal and maternal circulation that protects the offspring as well as the mother against later development of breast cancer.
Table 1

<table>
<thead>
<tr>
<th>Maternal Conditions</th>
<th>Maternal Serum AFP Concentration</th>
<th>Maternal Lifetime Breast Cancer Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

1a
- Pregnant vs. Non-pregnant: $1 > 2$ (20)*, $1 < 2$ (21)
- Pregnant, black vs. Pregnant, white: $1 > 2$ (22), $1 < 2$ (23)
- Pregnant, lean vs. Pregnant, obese: $1 > 2$ (22), $1 < 2$ (24)
- Pregnant, consuming no alcohol vs. Pregnant, consuming alcohol: $1 > 2$ (25), $1 < 2$ (26)

1b
- Pregnant, hypertensive vs. Pregnant, normotensive: $1 > 2$ (27), $1 < 2$ (28)
- Pregnant, with multiple fetuses vs. Pregnant, with a single fetus: $1 > 2$ (29), $1 < 2$ (30)
- Pregnant, fetus with neural tube defect vs. Pregnant, fetus no neural tube defect: $1 > 2$ (31), $1 < 2$ (32)

*The numbers in the brackets are the reference sources for the data.

Recently, Richardson et al. (34) have reported measuring this association directly. She found that the concentration of AFP in cryogenically stored maternal sera was inversely correlated to the risk of breast cancer in these mothers 20 to 30 years after their pregnancies. As mentioned earlier in this report, we speculate that the AFP which crosses the placenta and enters the maternal circulation extinguishes microscopic premalignant foci that later on in life would be promoted into clinically detectable breast cancers. Our own work has shown that administration of natural mouse AFP (10), natural human AFP (11), or recombinant human AFP (13) can inhibit estrogen-stimulated growth of human MCF-7 breast cancer xenografts.

In year one of this grant we focused our study on recombinant human AFP obtained from our collaborators at McGill University, who licensed the rights to their patent for producing AFP to a start-up biotech company, Atlantic Biopharmaceuticals. We tested several batches of their AFP and found variable activity. We believe the basis for this variability was the harsh conditions used to isolate the protein from the E. coli expression system. The protein was not secreted in this system. Rather, it was contained in inclusion bodies inside the E. coli. The chemical conditions required to lyse the E. coli and break the inclusion bodies denatured the protein. The resolubilized protein was diluted and allowed to refold but in some cases probably did not return to the activable conformation. In retrospect, an expression system which secreted the protein would be preferable, and we have taken advantage of this experience in year 2 of the grant. In
spite of these problems, an active batch was identified in our uterine bioassay screening procedure. This batch was activable to a form that was anti-estrogenic by estradiol, 13-cis retinoic acid and vitamin D_3 (calcitriol). The E_2-activated recombinant AFP was comparable to natural AFP isolated from human cord sera in its ability to stop the growth of human MCF-7 breast cancer xenografts (35). It did not inhibit the growth of estrogen-independent MDA-MB-231 human breast cancer xenografts. At this point, our colleagues at McGill University ran out of this batch of AFP. Atlantic Biopharmaceuticals was supporting production of this AFP and invested in scaled-up production of AFP at a GMP facility. Scale-up did not yield active batches of material. The company has withdrawn its support for AFP production. We are not hopeful that our colleagues at McGill will find another investor to support large-scale production of AFP by their methods. Concurrently, we were developing our own sources of AFP as a back-up as outlined in our original grant proposal. We produced domain III of recombinant human AFP in a baculovirus expression system and demonstrated that it was activable and retained the potency of the full-length molecule. We also purified AFP from a human hepatoma cell line, Hep G-2, and demonstrated that it was activable to a form that was anti-estrogenic. Progress in year 2 has occurred with these two preparations of AFP.

**BODY**

**a. Methods and Results Obtained**

Significant progress has been made with nhAFP from Hep G-2 cells and rhAFP Domain III from a baculovirus expression system. It is not clear to us at this time which product is more translatable to the clinic. Both have their advantages, and work in year 3 will clarify this issue. Progress with nhAFP will be described first.

When Hep G-2 cells are transferred from serum-containing medium to serum-free medium, the concentration of AFP secreted into the culture supernatant increases sevenfold (Fig. 1). AFP is 18% of the total protein in serum-free supernatant compared to less than 1% of the total protein in serum-containing supernatant. This result is due to not only increased secretion of AFP under serum-free conditions, but also to diminished levels of all other serum proteins except AFP in the serum-free supernatant.

Following concentration of crude supernatant using a 10,000 molecular weight cutoff, it was found that the supernatant alone contained antiuterotrophic activity (Fig. 2). Fractionation of the crude supernatant through an anti-AFP column indicated that the activity was in the AFP-containing fraction (Table 2). The activity titered out at 10 μg of AFP unless activation conditions were employed, in which case it titered out at 10 ng of AFP.

![Fig. 1 AFP Concentration in Supernatant of HEPG2 Cells vs. Time of Cells in Serum-Free Medium](image-url)
Table 2
Anti-uterotrophic Activity of AFP
Purified from Supernatant of Hep G-2 Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Inhibition of E2-Stimulated Growth of Mouse Uterus (Mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfractionated 100 µg AFP</td>
<td>45 ± 8</td>
</tr>
<tr>
<td>Anti-AFP Column 100 µg protein Non-adherent</td>
<td>0</td>
</tr>
<tr>
<td>Anti-AFP Column 100 µg AFP Adherent</td>
<td>45 ± 6</td>
</tr>
</tbody>
</table>

Fig. 2 Antiuterotrophic Activity of nhAFP

Fig. 3 Inhibition of MCF-7 Breast Cancer Xenograft Growth by nhAFP

The fact that higher doses of AFP were antiuterotrophic without prior exposure to activating ligands suggested that these conditions of higher dose might also have anti-breast cancer activity. This was tested against the human MCF-7 breast cancer growing as a xenograft in immune-deficient mice. As shown in Fig. 3, tumor did not grow in mice receiving 100 µg of AFP daily for one month beginning on the day of tumor implantation. The pattern was similar to that seen in mice not receiving the required amount of estrogen by Silastic estradiol implants (Si/E_2) to support the growth of tumor.

This dose of AFP also stopped the growth of tumors which had been allowed to seed and grow for 10 days prior to treatment. Blood levels of AFP and estradiol were obtained during treatment. The half-life of AFP was determined to be 27 hours, and the serum E_2 levels actually increased from 71 pg/ml to 124 pg/ml in mice receiving AFP. This is consistent with AFP blocking the feedback mechanism whereby E_2 stimulates the hypothalamic-pituitary axis to down-regulate the secretion of gonadotropins. As mentioned earlier, this syndrome is seen in patients with AFP-secreting hepatomas (17). On histological examination of tumors from AFP-treated mice, there was no change in vascularity, fibrosis, mucin, necrotic debris or inflammation. The appearance was consistent with a cytostatic effect of treatment in which natural cell death was unaffected while cell renewal was suppressed. There did appear to be a less invasive pattern in tumors from
Disulfide bonding pattern of human AFP and the amino acid sequence homology with mouse AFP and human albumin. The layout and the numbering of double loops are according to Brown (21). The comparisons of the amino acid sequences were made by aligning the disulfide bridges and maintaining the highest nucleotide sequence homology. The least number of gaps were introduced while maintaining the triplet codons. Amino acid residues homologous to those of mouse AFP or human albumin are indicated by blackening the amino acid circle above or below, respectively. Four amino acid residues missing in mouse AFP are indicated by stars. Arrows indicate potential N-glycosylation sites in human AFP (H), mouse AFP (M), and rat AFP (R).
AFP-treated mice, and this needs to be followed up and quantitated in future experiments. The proliferative index of the tumors was assessed using a Cell Analysis Systems-200 Image Analyzer. Cells in S phase of the cell cycle dropped from 38% to 10% as a result of AFP treatment. Correspondingly, cells in the G2G1 phase increased from 49% to 76% in the AFP group. These results are consistent with the histology results, which indicated that AFP interfered with renewal of tumor cells.

**Domain III AFP.** In year 1 we produced, isolated and tested a non-secreted Domain III AFP (Fig. 4) in a baculovirus expression system. This fragment was activable by incubation with E2, cis-retinoic acid and vitamin D3 and was comparable to full-length protein in antiuterotrophic activity. However, we found variability in activity among different batches of recombinant human Domain III AFP. The variability was similar to that found with full-length rhAFP obtained from our colleagues in Montreal. Thus, in year 2, we embarked on developing a recombinant system that would both produce and secrete Domain III AFP. We are currently utilizing the baculovirus expression system to produce a biologically active, secreted Domain III of AFP. Our current approach utilizes a new baculovirus transfer vector (pACSEC2T, Pharmingen) which incorporates an N-terminal leader sequence from the baculoviral protein gp67 (to facilitate secretion from insect cells), the Glutathione-S-Transferase (GST) protein from *Schistosoma japonicum* (to facilitate purification and solubilization of fusion protein) and Domain III of human AFP (Fig. 5). The transfer vector containing the coding sequence for Domain III of AFP has been cotransfected into SF9 insect cells to produce recombinant virus. Virus was then plaque-purified, screened for incorporation of Domain III coding sequence into the viral genome (PCR) and the ability to produce secreted recombinant protein (Western blot).

Virus was then amplified by three serial passages and titred by plaque assay. Production of secreted protein was demonstrated by Western blot analysis of cell culture medium, which was then loaded onto a glutathione-Agarose (Sigma) column and washed with PBS; the protein was eluted with 5 mM glutathione. A number of different cell lines and cell culture conditions were evaluated in terms of their ability to produce and secrete GST-DIII. Progress on this study has led to conditions amenable to producing large quantities of pure protein: Recombinant virus was added to SF9 insect cells and allowed to infect the cells. The medium was then aspirated and the cells were covered with Graces medium (Gibco) containing only bicarbonate buffer, pH 6.0. Protein was isolated at levels at least five times that of any of previous conditions we have evaluated. Most importantly, the Domain III protein produced in this system was biologically active. When evaluated in the immature mouse uterine growth bioassay, the Domain III protein (cleaved by thrombin from GST-DIII protein bound to glutathione-Agarose resin), upon activation with E2, inhibited estrogen-stimulated growth by 38%-40% but apparently lost activity over time (7-10 days when stored in solution at +4°C). If these preparations were then treated with 5 mM glutathione prior to injection, the activity was recovered. We are in the process of
scaling up production of this material from microgram quantities to milligram quantities so that we can determine whether at higher doses this material is active without prior exposure to ligand.

There are two very important separate side projects which are in progress using, for now, limited intramural funding. These projects were made possible by the progress which occurred in year 2 of this grant. Both projects have been submitted for extramural funding. [1] My colleague and coinvestigator on this grant, Dr. Thomas Andersen, who has made Domain III AFP, is currently working on making the subdomains (a, b, c) of Domain III AFP (Fig. 4) in an attempt to isolate the active site of this protein. He is Director of our Peptide Synthesis Facility and is hopeful that he will find activity in a small enough part of this protein that this part can be synthesized as a peptide. It is hypothesized that the peptide will not require exposure to ligand for activation. This is consistent with our theory that the role of activating ligand is to cause a conformational change in the protein which exposes the active site. He has made subdomain IIIab and has found antiuterotrophic activity which required exposure to E₂ ligand. One of our former colleagues, Dr. Gerald Mizejewski, has recently reported on a peptide that he made from Domain IIIb of the molecule which had activity at nanogram quantities and did not need activation (36). Dr. Andersen has subsequently produced 300 mg of this peptide, and we have found antiuterotrophic activity at the 100-μg level (no ligand-induced activation required) but no activity at the nanogram level as reported by Mizejewski et al. (36). This is still being investigated, but certainly adds credence to the active peptide theory.

[2] My colleague, Dr. Bruce Line, who is Chief of Nuclear Medicine at Albany Medical College, has labeled our AFP preparations with technetium-99m. Together we have found that Tc-99m hAFP localizes in and images human breast cancer xenografts more effectively than the agents currently in clinical trial for this purpose (see abstracts in Bibliography of this report). Dr. Line submitted a grant proposal to the U.S. Army Breast Cancer Program on September 1, 1995 to develop Tc-99m AFP as a breast cancer imaging agent. It received a very favorable scientific review (top 8%). However, it was not funded based on programmatic review. He has resubmitted a revised grant to the Army on this topic for the July 17, 1996 deadline and is hopeful this will pass both scientific and programmatic review. His proposal has extremely high relevance to breast cancer detection and is very translatable to the clinic.
CONCLUSIONS AND FUTURE WORK

Increasing the dose of AFP exempts the protein from the ligand-induced activation requirement and simplifies its use for therapeutics. Two explanations which could account for this outcome are as follows.

1. The untransformed molecule is intrinsically only 1% as active as the transformed molecule and requires a 100-fold increase in dose to achieve comparable activity.
2. There are a small percentage of molecules already in the active form and increasing the dose loads the receptors with active molecule.

Under both of these explanations, adding ligand produces an electrochemical environment which influences the conformation of the molecule and converts a significant percentage of the molecules to the active form. However, with dose adjustments the same level of antiestrogenic activity is achieved, whether uninfluenced or ligand-influenced AFP is used. Thus, it seems reasonable to use the simpler formulation (untransformed AFP) as long as toxicity is not an issue. It appears from our xenograft data that toxicity is not an issue, which is consistent with the fact that even at these higher AFP doses, they result in plasma levels of AFP which are still well below those found during fetal life (1).

Domain III rhAFP retains the activity found in the full-length molecule. We now have a baculovirus expression system that secretes good quantities of Domain III rhAFP into the supernatant of SF9 cells. Moreover, the purification strategies that we have worked out have been specifically designed so that only gentle, physiological conditions are used throughout the harvest of the protein. This improves the yield of active AFP, since we know that harsh chemical conditions compromise the activity of this protein. It is reasonable to expect that Domain III rhAFP will behave like the natural full-length protein and be active in the untransformed state as long as higher doses are employed.

During the third year of this grant we will continue to produce and purify full-length natural hAFP and Domain III rhAFP. Aims 2, 3, and 4 will be completed with the 100-μg dose of full-length natural hAFP. As domains, subdomains and peptides continue to evolve, they will be compared to full-length natural hAFP in their activity. We will evaluate other estrogen-receptor-positive and estrogen-receptor-negative breast and non-breast tumors for sensitivity to the 100-μg dose of untransformed natural hAFP. We will complete the development of a receptor assay for AFP and use this assay to quantitate AFP receptor on tumors that are sensitive or resistant to growth inhibition by AFP. We will quantitate serum FSH and E2 levels in mice whose tumors are regressing in response to AFP, since these appear to be sensitive intermediate markers of AFP activity in vivo. We will continue to evaluate histological, cell cycle and apoptotic changes that occur in tumors which are growth-inhibited by AFP.
REFERENCES


BIBLIOGRAPHY


Jacobson, H. I., Andersen, T. T., and Bennett, J. A. Transformed AFP (tAFP) in pregnant women mediates their reduced breast cancer risk. Tumor Biol 1995; 16:131-132.


**PERSONNEL RECEIVING PAY**

<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>James A. Bennett, Ph.D.</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>Herbert I. Jacobson, Ph.D.</td>
<td>Coinvestigator</td>
</tr>
<tr>
<td>Thomas T. Andersen, Ph.D.</td>
<td>Coinvestigator</td>
</tr>
<tr>
<td>Theresa Kellom, Ph.D.</td>
<td>Coinvestigator</td>
</tr>
<tr>
<td>ShuJi Zhu</td>
<td>Research Associate</td>
</tr>
<tr>
<td>Andrea Mirarchi</td>
<td>Research Technician</td>
</tr>
</tbody>
</table>
MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCP, 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 technical reports written for the following contracts. Request the limited distribution statement for these contracts be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

<table>
<thead>
<tr>
<th>Contract Number</th>
<th>Accession Document Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAMD17-94-J-4030</td>
<td>ADB215484 ✓</td>
</tr>
<tr>
<td>DAMD17-94-J-4138</td>
<td>ADB215863</td>
</tr>
<tr>
<td>DAMD17-94-J-4158</td>
<td>ADB215553</td>
</tr>
<tr>
<td>DAMD17-94-J-4278</td>
<td>ADB215864 ✓</td>
</tr>
<tr>
<td>DAMD17-94-J-4267</td>
<td>ADB216187 ✓</td>
</tr>
<tr>
<td>DAMD17-94-J-4200</td>
<td>ADB216054</td>
</tr>
<tr>
<td>DAMD17-94-J-4185</td>
<td>ADB219284</td>
</tr>
<tr>
<td>DAMD17-94-J-4172</td>
<td>ADB224562 ✓</td>
</tr>
<tr>
<td>DAMD17-94-J-4156</td>
<td>ADB216186</td>
</tr>
<tr>
<td>DAMD17-94-J-4082</td>
<td>ADB215979</td>
</tr>
<tr>
<td>DAMD17-94-J-4053</td>
<td>ADB216052</td>
</tr>
<tr>
<td>DAMD17-94-J-4028</td>
<td>ADB218953</td>
</tr>
</tbody>
</table>

2. Point of contact for this request is Ms. Betty Nelson at DSN 343-7328 or email: betty_nelson@ftdetrck-ccmail.army.mil.

FOR THE COMMANDER:

[Signature]

PHYLIS M. BINEHART
Deputy Chief of Staff for Information Management