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PRINCIPAL INVESTIGATOR: Paul F. Engstrom, M.D.

CONTRACTING ORGANIZATION: Fox Chase Cancer Center
Philadelphia, Pennsylvania 19111

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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Philadelphia, Pennsylvania 19111

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The goals of this research are to develop interdisciplinary studies of the etiology, biology and prevention of ovarian cancer. Three projects make up this program: Project I: Ovarian Cancer Consortium for Research and Surveillance, which will provide geographically-based system for recruitment, evaluation, surveillance and management of women at high risk for ovarian cancer. Project II: Facilitating Decision-Making About Prophylactic Oophorectomy is a behavioral research study in women at high risk for ovarian cancer who are considering or electing prophylactic oophorectomy. Project III: This Phase II Chemoprevention Study of Ovarian Cancer will evaluate the effect of fenretinide (4HPR) versus a placebo on intermediate or surrogate biomarkers of ovarian neoplastic progression in the ovaries of high risk women who have elected prophylactic oophorectomy.

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Paul F Engstrom
Principal Investigator's Signature

10/25/99
Date

Table of Contents

ANNUAL REPORT

PROJECT I
OVARIAN CANCER CONSORTIUM
FOR RESEARCH AND SURVEILLANCE

PROJECT DIRECTOR: MARY B. DALY, M.D., PH.D.
FOX CHASE CANCER CENTER

INTRODUCTION

Ovarian cancer is the leading cause of death from a gynecologic malignancy among women in the United States, and ranks second in incidence among gynecologic malignancies. Following years of limited progress, the excitement generated by the recent genetic-based insights into the process of ovarian carcinogenesis has fostered a truly unique spirit of collaboration within the scientific community to address complex research questions. Fox Chase Cancer Center (FCCC) has established The Ovarian Cancer Consortium for Research and Surveillance (OCCRS), a research-based infrastructure to facilitate the conduct of translational research, to promote rapid communication of relevant findings to the professional and lay communities, and to transfer novel prevention, screening and treatment strategies into clinical practice. The OCCRS utilizes the resources of FCCC, Cooper Medical Center, Bowman Gray Medical School, and Reading Medical Center to recruit families with one or more cases of ovarian cancer. Extensive information on medical, reproductive, family and lifestyle factors are being collected using standardized questionnaires. Samples of blood from all participants as well as tissue specimens from affected individuals and those undergoing prophylactic oophorectomy or surgery for other gynecologic conditions, are being collected and stored in a centralized tissue specimen bank at FCCC to serve as a resource for future research. Counseling and surveillance protocols are being standardized for application at each site. The development of an ovarian cancer symptom checklist which can be piloted in a prospective fashion to identify a syndrome or set of syndromes which may prompt efforts at early detection is underway. The creation of this dynamic ovarian cancer research data base is providing a unique opportunity to address multidisciplinary research questions and to work with providers and public health advocates to bring the knowledge about ovarian cancer generated by the genetic revolution to the community.

BODY

The operational premise for establishing the OCCRS in Year One was to integrate the study aims into the existing research bases at the collaborative institutions. These institutions' various screening and treatment programs provide an infrastructure readily adaptable to recruiting both high-risk individuals and ovarian cancer patients into this research. The following describes the progress during Year One associated with each Task outlined in the Statement of Work.

Task 1. Development and Implementation of a Recruitment Strategy – Months 1-6.

a. Key personnel have been identified at each site and may be found in **Appendix A.**
b. Three different telephone scripts have been developed and implemented to recruit ovarian cancer patients, relatives, and high-risk individuals. **(See Appendix B)**
c. A method to identify ovarian cancer patients has been implemented at FCCC. Primary care nurses of staff medical and gynecological oncologists work with the FRAP staff to target appropriate patients. The Project Manager then contacts them using a telephone script and a meeting is arranged in conjunction with a clinic appointment. High-risk individuals are identified as relatives of the patients and through the established FRAP protocol. The staff involved has extensive experience in recruiting individuals to numerous studies and adaptation to the OCCRS requirements has been a fluid process. The staffs at both Reading Hospital and Medical Center and Cooper Hospital/University Medical Center have experience working with FCCC in previous studies and the mechanisms of recruitment to the OCCRS are established. Protocols have received IRB approval.
The Project Manager provided onsite training of targeted staff at Bowman Gray Medical School. Integration into established patterns of identifying high-risk women through the Epidemiology Section of their Department of Public Health Sciences is underway.

d. Marketing emphases of the OCCRS at FCCC in Year One include identifying the target population and developing a program brochure. Staff has interfaced with hospital nursing personnel to educate and promote the project. The Public Relations Director has been kept apprised of the status of the project and has generated press releases. Participation in local and national ovarian cancer advocacy organizations has provided opportunities to exhibit cancer prevention and study recruitment materials.
e. A procedure manual is complete and has been provided to the Network sites.
f. Recruitment of participants is actively underway at FCCC with a total of 71 women accrued to date. Implementation of the accrual process at Bowman Gray will begin within the next three months upon application approval by the General Clinical Research Center (GCRC), the clinical research support area onsite. A turnover in available staff to support the study has deterred the initial recruitment at Cooper Hospital but the process should be underway by the end of 1999. Eligible participants have been identified at Reading Hospital but medical emergencies experienced by the two key personnel have delayed enrollment.

Task 2. Establishment of a Computerized Data Base – Months 1-6

a. The data collection instruments including Health History Questionnaire, Miller Behavioral Style Scale (MBSS) and Recent Impact of Events (RIES) psychological surveys and Willet diet questionnaire were piloted through the FRAP program and required no adaptation. A treatment questionnaire for ovarian cancer patients has been developed and, pending IRB approval, will be reviewed after the first 10 pilot responses are returned. **(See Appendix C)**

Task 2: Establishment of a Computerized Data Base – Months 1-6 (Continued)

b. Mr. Andrew Balshem and Mr. John Malick have designed and built the research database using the ORACLE suite of products. Staff at the participating sites have been trained in the forwarding of completed hardcopy data collection instruments to FCCC for data entry.
c. Ms. Honey Salador and Ms. Rose Batson have primary responsibility at FCCC for input into data screens.
d. Quality assurance is maintained by careful oversight of data as it is received and entered in a timely fashion. The necessary checks to link data between tables and within tables to assure accuracy are in place and are run twice weekly.
e. A dictionary for our most comprehensive data collection tool, the Health History Questionnaire, is in place.

Task 3: Development of Informed Consent Practice – Months 1-4

a. Initial consent of participants is obtained by phone at the time of initial contact. Arrangements are made to meet in person prior to blood sampling to review at length a participant consent form. Key components of the document include study purpose, procedures, contacting of family members, use of specimens for future cancer studies, registry rights, benefits, risks and costs of participation, confidentiality, and options for withdrawal and termination. After addressing all questions from the participant the consent form is signed and witnessed and a copy provided for the client's records. **(See Appendix D)**

Task 4: Establishment of an Ovarian Cancer Tissue Bank – Months 1-6

a. Well-developed protocols for collecting blood and tissue specimens were already developed through the FRAP at FCCC and were easily adapted to the requirements of this study.
b. Technical staff are in place and are actively preparing, labeling and storing biospecimens. A pathology review form has been designed.
c. The staffs at Cooper Medical Center and Reading Hospital and Medical Center are fully trained and have experience in biospecimen collection. A detailed procedure has been reviewed with the project manager at Bowman Gray but has not yet been implemented pending IRB and GCRC approval. The laboratory personnel in the GCRC are highly skilled in enabling specimen collection according to research protocols.
d. Blood collection supplies have been provided to all sites.
e. All software for entering specimen information into the database has been developed and is in use.
f. Biospecimen collection was initiated at FCCC in April 1999 (month 6) and is actively underway. Collection at the network sites is expected by December 1999.

Task 5: Development and Implementation of Symptom Checklist

Preliminary planning for this project has taken place and we are in the development phase. We are collaborating with Andrea Barsevick, R.N., D.N.Sc., Director of Nursing Research at FCCC, to design, pilot and implement a symptom checklist. We will work with the primary care nurses of the gynecological oncologists to identify women recently (within one year) diagnosed with ovarian cancer to conduct interviews. Results of the interviews will be used to create a symptom checklist to pilot among 50 women participating in a high risk screening program. Upon revision the finalized instrument will be incorporated into the screening protocol and administered annually in a prospective fashion.

Task 6: Standardization of Genetic Risk Counseling Protocols – Months 1-6

a. A comprehensive genetic risk counseling protocol involving a risk assessment team approach is well established by the Margaret Dyson Family Risk Assessment Program at FCCC. This program includes education, risk assessment and cancer risk counseling. Education focuses on reproductive anatomy, cancer risk factors, introduction to cancer genetics and genetic testing, and prevention strategies. Participants receive an educational booklet designed with culturally sensitive graphics in a three-ring binder format to allow ease of updating information. A nation-wide referral pattern is established and utilized by our genetic counseling staff.
b. The standardized protocol for cancer risk counseling includes assessment and monitoring of outcomes through pre and post-test measurement.
c. The FRAP at FCCC has been the model for the similar risk evaluation programs at Cooper Medical Center and Reading Hospital and Medical Center and staff have been trained. Collaboration with a certified genetic counselor at Bowman Gray Medical Center is underway to assure consistency in content of counseling to be provided at that site.
d. Counseling is underway at FCCC and 50 women have received consultation. The identified personnel at the other sites are providing counseling for a number of other cancer risk studies and anticipate implementing this study by December 1999.

Task 7: Develop a Comprehensive Education Program for Providers and Participants –Months 1-36

Sophisticated educational tools already in place in the FRAP at FCCC include a compact disc-interactive (CD-i) format and three-ring binder booklets on ovarian and breast cancer risk. The Project Manager has begun researching available ovarian cancer risk educational materials by networking with other risk evaluation programs, professional oncology nursing colleagues, and internet options. Significant time will be devoted in Year Two to develop a plan for designing educational videos.

KEY RESEARCH ACCOMPLISHMENTS

- Three to four tubes of blood, approximately 10 ml each, were collected from 48 women eligible to participate in the OCCRS registry. Due to the sensitive nature of family and personal medical information, we take extensive precautions to protect the privacy of participants. Each participant is given a unique study code which is the only source of identification visible on the blood samples.

- Lymphocytes were isolated from one to two tubes of blood by Ficoll-Paque centrifugation, divided into four to eight aliquots, and cryopreserved in liquid nitrogen for future transformation or nucleic acid preparation.

- Genomic DNA was isolated from one tube of blood and is being stored at 4°C.

- Plasma and whole blood (i.e., blood spots) were also banked and are being stored at –80°C and room temperature, respectively.

- Thirty-one constitutive DNAs from registry participants have tested for recurrent mutations in *BRCA1* and *BRCA2* by a heteroduplex mobility assay (HMA), however no mutations have been identified.

- Two additional DNA samples were tested for mutations in the *BRCA1* gene by an enzyme mutation detection assay (EMD). One mutation in exon 11, 3600del11, was identified in an unaffected 39 year old female whose mother was diagnosed with breast and ovarian cancers at age 40. Pedigree analysis shows other ovarian (age 66), and breast cancers (ages 60 and 49), as well as cancers of the colon, pancreas, and skin. Ethnicity has been reported as Italian (Catholic) and German (Catholic). The second mutation, also in exon 11 (2012insT), was identified in an unaffected 23 year old female whose mother was diagnosed with ovarian cancer at age 50 and whose paternal grandmother was diagnosed with ovarian cancer at an unknown age. Maternal aunts were diagnosed with cancers, one with bone and breast at unknown ages, and another with breast cancer at age 26. Pedigree analysis identifies a maternal grandmother also was diagnosed with both bone and breast at unknown ages, and a paternal grandfather with leukemia also at an unknown age. Ethnicity of the family is not available at the time of this report.

- Thirty-two ovarian tumor samples were obtained from consenting patients undergoing surgery at FCCC from 1998 to present. A portion of the debulked tumor mass was used immediately for DNA isolation, while the remaining tumor samples are being stored at -70°C.

- Twenty-nine blood samples, corresponding to the patients undergoing ovarian cancer surgery at FCCC were collected and DNA isolated. Ovarian tumor DNA and corresponding normal (from peripheral blood) are being used to evaluate non-random chromosome 17 loss in ovarian cancer (as part of **Project I).**

- Sixty-two archival ovarian tumor specimens were collected through the Pathology Department at FCCC and tissue sections were obtained (15 slides per specimen).

- Seven ovaries removed for prophylaxis were collected and processed. Portions of each oophorectomy specimen were used to establish primary cultures of surface epithelial cells. The remaining tissues were fixed, embedded, and sectioned for histologic evaluation (as outlined in **Project III**).

- Archival ovarian tissue specimens removed for prophylaxis from women considered to be at increased risk for the disease were forwarded to Dr. A. Klein-Szanto for histological analysis (as outlined in **Project III**).

- Prophylactic ovarian sections containing preneoplastic lesions were evaluated for cell proliferation by immunohistochemistry using antibodies against Mib1 (as outlined in **Project III**).

REPORTABLE OUTCOMES

Manuscripts

Bruening, W., Prowse, A.H., Schultz, D.C., Holgado-Madruga, M., Wong, A., Godwin, A.K. Expression of OVCA1, a candidate tumor suppressor gene, is reduced in tumors and inhibits growth of ovarian cancer cells. Cancer Research, in press, 1999.

Serum repositories:

We are storing serum as indicated in the section on Key Research Accomplishments.

Cell lines:

Primary surface epithelial cell cultures were initiated from ovaries removed for prophylaxis from seven women. These cell lines are being used to study the effect of fenretinide on cell growth and gene expression patterns using cDNA microarray chips.

CONCLUSIONS

We have collected and banked lymphocytes, DNA, blood plasma, and whole blood from 48 women willing to participate in the OCCRS registry. We have also obtained fresh-frozen (32 specimens) and archival (62) tissue specimens. DNA has been obtained from the fresh-frozen specimens and corresponding blood samples. Two laboratories at FCCC (Drs. R. Raftogianis and A. Yeung), which are not part of the program grant, have already approached the registry to obtain DNA samples. Full gene analysis of *BRCA1* in two unaffected individuals with a family history of ovarian cancer resulted in the identification of two deleterious mutations. Therefore, these women are eligible to participate in the studies proposed in Project II "Facilitating decision making about prophylactic oophorectomy" and Project III "Phase II chemoprevention study of ovarian cancer". Archival ovarian tissue specimens removed for prophylaxis from women considered to be at increased risk for the disease have been collected and evaluated for early stage tumors and preneoplastic lesions. Immunhistochemical studies have been performed to determine if the epithelial cells forming these lesions are proliferating abnormally.

REFERENCES

To date there are no pertinent references to this report.

ANNUAL REPORT

PROJECT II
FACILITATING DECISION MAKING ABOUT
PROPHYLACTIC OOPHORECTOMY

PROJECT DIRECTOR: SUZANNE M. MILLER, PH.D.
FOX CHASE CANCER CENTER

Introduction

Project II, *Facilitating Decision-Making about Prophylactic Oophorectomy* focuses on how women with a familial risk of ovarian cancer make decisions regarding their preventative options, specifically prophylactic oophorectomy (surgical removal of the ovaries). The primary goal of the study is to explore the psychological factors that influence a woman's decision to undergo or forego the procedure. A secondary goal is to identify whether high monitors (who typically scan for and exaggerate cancer threats) show a different pattern of response than low monitors (who typically distract from and minimize health threats). Data obtained from this study will be used to develop an enhanced counseling intervention to facilitate decision-making and maximize patient adjustment. At the end of year II a pilot study will be designed and conducted to provide a preliminary evaluation of the feasibility and efficacy of the enhanced counseling intervention.

Body

During the initial year of funding, considerable attention has been devoted to careful start-up efforts. Institutional Review Boards from Fox Chase Cancer Center (FCCC) and Reading Hospital have approved the research protocol. The IRB of Cooper Hospital/University Medical Center (CH/UMC) has approved the protocol, but is waiting for assurance from the Office for Protection from Research Risk (OPRR). Graduate Hospital's IRB is still reviewing the protocol, with a response expected within the next month. Modifications were made to the research protocol in order to ease participant burden and to clarify requirements and directions. Specific refinements include:

- The original schedule of surveys included a baseline measure with follow up measures at 1 week, 6 weeks, 6 months, and 12 months following a participant's education session. This has been amended to consist of a baseline measure with follow-up measures in 3 months, 6 months, and 12 months. This amendment was made to reduce participant burden by spacing out the assessments.

- The format of the assessments has been changed from self-report questionnaires with interviews to self-report questionnaires only. All assessments will be conducted via questionnaire mailings. This change should improve our ability to recruit and retain participants by providing a more private, less intrusive method of data collection.

- An introduction to the study and directions for each questionnaire has been added or enhanced, since surveys will be completed at home without an interviewer.

- Eligibility criteria has been modified from women ages 25-60 with two first or second degree relatives diagnosed with ovarian cancer to women ages 18 and older with at least one first- or second-degree relative diagnosed with ovarian cancer, in order to provide a better representation of the population interested in prophylactic oophorectomy.

In addition a procedural plan was designed to ensure consistency in dealing with multiple sites. This entails identifying key personnel, developing a standardized protocol to contact potential participants, and the establishment of a computerized database for all study data. A series of meetings held between staff at FCCC and contacts at collaborating sites enabled us to systematically develop and enact this plan.

At each site a key contact is responsible for initiating contact with eligible individuals. Individuals who express an interest in the research are directed to Ms. Maggie Longacre at Fox Chase Cancer Center, who was hired in August of 1999 to assist Dr. Suzanne Miller and Dr. Carolyn Fang with the supervision of the project. Ms. Longacre will contact eligible participants using a standardized telephone recruitment script. Individuals recruited from FCCC and Reading Hospital will receive all four packets of questionnaires via mail. At Cooper and Graduate Hospitals the study's baseline packet of questionnaires will be distributed to participants during clinic hours, while the remaining sets of questionnaires will be sent out through the mail. All packets of questionnaires include self-addressed postage-paid envelopes for the participants to return their completed questionnaires.

These start-up efforts have ensured the effective recruitment and retention of participants. Consistent with our goal of establishing an overarching database to encompass all three projects, meetings were arranged with FCCC's Senior Research Biostatistician and his staff to develop this database for the storage and analysis of study data.

Key Research Accomplishments

- A review and analysis of the literature on decision-making about prophylactic oophorectomy was conducted. This review paper, *Decision Making about Prophylactic Oophorectomy among At-Risk Women: Psychological Influences and Implications* by Suzanne M. Miller, Ph.D., Carolyn Y. Fang, Ph.D., Sharon L. Manne, Ph.D., Paul F. Engstrom, M.D., and Mary B. Daly, M.D., Ph.D. is currently in press in *Gynecologic Oncology.*

- Implementation of study protocol and initiation of recruitment efforts.

- Completion of pilot studies investigating the predictors of women's intentions to undergo prophylactic oophorectomy. One empirical paper has been submitted by Karen E. Hurley, Ph.D., Suzanne M. Miller, Ph.D., Josephine W. Costalas, MS, Mary B. Daly, MD, Ph.D. for publication, entitled *Anxiety/Uncertainty Reduction as a Motivation for Interest in Prophylactic Oophorectomy in Women with a Family History of Ovarian Cancer.* This study investigated the relation of cancer anxiety and other factors to interest in prophylactic oophorectomy in a group of women with varying degrees of familial risk for ovarian cancer. Another empirical paper, *The Influence of Attentional Style and Risk Perceptions on Intentions to Undergo Prophylactic Oophorectomy Among FDRs*, by Carolyn Y. Fang, Suzanne M. Miller, Mary B. Daly, and Karen Hurley, is almost ready for submission. This paper illustrates the impact of monitoring attentional style and perceived risk on at-risk women's intentions to undergo prophylactic oophorectomy.

Reportable Outcomes

Especially relevant in the pilot work, the predictors of women's intentions to undergo prophylactic oophorectomy were investigated. Eighty female first-degree relatives (FDRs) of ovarian cancer patients completed the measure high monitor/low monitor upon enrollment into a Family Risk Assessment Program. Following participation, measures of cancer risk perceptions, perceived benefits and cost of surgery, and intentions to undergo preventative surgery were obtained. Hierarchical regression analyses indicated that a high monitoring attentional style was associated with greater intentions to undergo prophylactic oophorectomy. In addition, perceived risk and perceived benefits of the procedure were positively associated with intentions to undergo

preventive surgery. Finally, a significant interaction between attentional style and perceived risk revealed that high monitors who felt at an increased risk for ovarian cancer were less inclined to undergo surgery, whereas low monitors who perceived themselves to be at increased risk were more inclined to undergo surgery.

In addition, in depth information was obtained from two study participants recruited from FCCC Family Risk Assessment Program (FRAP). Specifically we investigated ovarian cancer knowledge in terms of screening and prevention, ovarian cancer beliefs, emotional status, and decision regarding prophylactic oophorectomy. Participant I is a 46-year-old female who has a first-degree relative (mother) with ovarian cancer. Participant II is a 48 year-old woman who has two first-degree relatives and one second-degree relative diagnosed with cancer. Her mother was diagnosed with cervical cancer, her sister was diagnosed at age 35 with breast and uterine cancer, and her aunt (mother's sister) was diagnosed with ovarian cancer at age 50. The following data were obtained from each participant's baseline packet of questionnaires.

Ovarian Cancer Knowledge, Screening, and Prevention
Participant I answered 17 out of 21 questions (81%) correctly on the Ovarian Cancer Knowledge survey, while participant II answered 16 out of 21 questions (76%) correctly. These results confirm that both participants have a high level of knowledge regarding ovarian cancer. Participant I's screening recommendations came from the woman's doctor, which she valued as "extremely important." The screening and risk reduction recommendations presented by her doctor included oral contraceptives, pelvic exams, abdominal ultrasounds, and transvaginal ultrasounds. In addition, the participant identified abdominal ultrasound and transvaginal ultrasound as "very effective" at detecting ovarian cancer, while pelvic examinations and CA-125 blood tests were selected as somewhat effective and moderately effective, respectively. Participant II's screening and risk reduction behaviors recommended by the participant's doctor, which she valued as being "quite a bit" important, included pelvic exams and abdominal ultrasounds.

Ovarian Cancer Beliefs
Participant I perceived her level of risk to be about the same in comparison to women who also have close relatives with ovarian cancer. Based on the participants level of risk, items believed to be "moderately" or "very" effective in preventing ovarian cancer include childbearing, eating a low fat diet, and taking oral contraceptives. Prophylactic oophorectomy was the only item selected as being "completely" effective.

Participant II rated her risk for developing ovarian cancer to be a little higher compared to women her own age who also have an increased family risk of developing cancer. She rated her chances of developing ovarian cancer to be 50%, with or without taking oral contraceptives. Oral contraceptives were valued by the participant to be "somewhat effective" in preventing ovarian cancer. She is currently not taking such a preventative measure, but does show interest in learning more regarding the possible effect oral contraceptives have on ovarian cancer. Prophylactic oophorectomy was also valued by this participant as a "completely effective" way to reduce ovarian cancer risk.

Coping/Affective Factors
From answering the SHORT COPE measure, which addresses self-regulatory coping skills, participant I revealed that she has been focusing a little bit on steps to take regarding her

situation. The Profile Of Mood States (POMS) measure indicated that in the last week she felt moderately lively, active, energetic, efficient, and full of pep, with no feelings at all of bad temperament, anger, gloominess, exhaust, or nervousness. The Monitoring Blunting Social Scale (MBSS) was also evaluated, which assesses the attentional monitoring style of individuals through interpreting selected responses regarding situations. Based on the MBSS scale this participant is identified as a high monitor.

Based on answers from participant II's completed SHORT COPE measure, this participant has been focusing a lot on problem-focused strategies and steps to take regarding her increased risk. The results of the participant's MBSS measure qualify her as a low monitor. According to her POMS measure she considers herself to be "moderately" active and energetic and only a "little bit" discouraged or anxious.

Current Decision Regarding Prophylactic Oophorectomy

At this point in the study participant I is confident that she will not undergo prophylactic oophorectomy within the next six to twelve months. The participant indicated possible reasons for and against surgery on the Pros and Cons survey. Possible reasons, as indicated by the participant, in favor of surgery included fear of getting cancer and the difficulty of early detection. Possible reasons against surgery included believing that there was too low of a risk for ovarian cancer, having a continuing interest in childbearing, and the perception of risk being associated with surgery.

Similarly, participant II is confident that she will not undergo prophylactic oophorectomy in the next six to twelve months, and she is "somewhat" confident she will make the correct decision regarding surgery. Based on the Pros and Cons survey, the participant neither agreed nor disagreed with any of the statements favoring prophylactic oophorectomy, but she did disagree with seven out of eight reasons against surgery. Statements she disagreed with included not having a high enough risk, not wanting to take hormone replacement therapy, having a doctor who recommended against it, feelings of being less feminine, and fear of revealing risk status to insurance carrier or employer.

Conclusion

This research will fill a void in the ovarian cancer risk literature. Women with an increased risk of ovarian cancer face a difficult decision regarding preventative surgery, and few resources are available to help them with their decision. Hence, it is important to explore factors associated with decision-making and to use the information to develop effective counseling interventions. Data from the preliminary study and the two participants reveal that cognitive and affective factors can impact decision-making. Through more systematic investigation of these factors, we will be able to develop a profile of decision making that will be used to design an enhanced counseling intervention. A pilot study will then investigate the effectiveness of the resulting counseling intervention.

References

None.

ANNUAL REPORT

PROJECT III
PHASE II CHEMOPREVENTION STUDY
OF OVARIAN CANCER

PROJECT DIRECTOR: ROBERT OZOLS, M.D., PH.D.
FOX CHASE CANCER CENTER

INTRODUCTION

While there has been progress in the treatment of ovarian cancer, most patients still die of this disease. High-risk individuals, by virtue of a strong family history of ovarian cancer and genetic analysis, frequently undergo a prophylactic oophorectomy (1). However, such a procedure is not always protective of a subsequent peritoneal carcinomatosis and is associated with significant morbidity, including the need for lifelong hormone replacement therapy. Evaluation of potential chemopreventive agents in ovarian cancer has been limited due to previous difficulties in identifying premalignant lesions and surrogate endpoint biomarkers (SEBs).

Fenretinide, a retinamide derivative of vitamin A, is a promising chemopreventive agent which induces apoptosis and decreases cell proliferation (2-6). It has an inhibitory effect on the growth of ovarian cancer cells and surface epithelial cells of the ovary (7). This research study tests the hypothesis that treatment of high-risk individuals with fenretinide will change the histologic features associated with a preneoplastic phenotype in ovaries as well as alter putative biomarkers of preneoplasia. To test our hypothesis we are conducting a Phase II clinical trial of fenretinide versus a placebo in women with high risk of developing ovarian cancer and a desire to undergo oophorectomy for prophylaxis. At the completion of the treatment phase of the clinical trial, all patients will undergo oophorectomy, and the histologic characteristics of the ovaries from the two groups of patients will be compared as well as the relative abundance of markers of cell proliferation and apoptosis. In addition, these results will be compared to ovaries removed from untreated individuals at no increased risk for ovarian cancer. This study will establish baseline values of SEBs in high-risk and normal-risk populations as well as evaluate the specific effect of fenretinide treatment on cell proliferation and apoptosis in precursor lesions of an ovarian cancer-prone population.

BODY

In May 1998, the Department of Defense notified the Fox Chase Cancer Center (FCCC) of its recommendation to fund our clinical prevention trial "Evaluation of Fenretinide as a Chemopreventive Agent for Ovarian Cancer." The study was submitted to the Fox Chase Cancer Center Research Review Committee (RRC) in June 1998. This committee reviews proposed clinical studies from the perspective of scientific rationale, study design, feasibility and conduct, patient registration and data management, statistical appropriateness and institutional priority. Additional information and revisions were requested by the RRC. Following institution of these changes, the study was approved by the RRC and submitted to the Institutional Review Board (IRB).

In August 1998, this IRB-approved clinical trial was reviewed by the Surgeon General's Human Subjects Research Review Board (HSRRB). Additional clarifications were requested and instituted. Approval was granted.

In February 1999, this study underwent review by the National Cancer Institute, Chemoprevention Branch (NCI, CB). The NCI, CB is very supportive of this study and is providing fenretinide as well as placebo. The NCI has certain responsibilities as Sponsor for the Investigational New Drug application (IND) of fenretinide. In order for the NCI, CB to fulfill its responsibilities, the protocol, associated case report forms, and consent were revised for submission to the Federal Drug Administration (FDA) as part of the fenretinide IND application.

In June 1999, this study underwent review and approval by the FDA as part of the fenretinide IND application.

Following each review and amendment, copies of the research protocol were submitted and approved by RRC, IRB, DOD and NCI. The approved study concept is as follows. This Phase II, double-blind, placebo-controlled trial will evaluate the potential effects of fenretinide (4-HPR) (*N*-(4-hydroxyphenyl)retinamide) in women at increased risk for ovarian cancer. A total of 71 participants (this includes a 10% "drop-out" rate) will be randomized to allow 32 evaluable participants per arm. Eligible to participate are women greater than 18 years of age who have decided to undergo a prophylactic oophorectomy due to increased risk for ovarian cancer defined by: 1) evidence of a genetic defect in BRCA1 or BRCA2, or 2) one or more first-degree relatives diagnosed with ovarian cancer prior to the age of 50 years, or 3) other family history contributing to risk: one first-degree relative diagnosed with ovarian cancer at any age and at least one other first- or second-degree relative diagnosed with ovarian cancer at any age. Participants will be randomized to take daily oral doses of either 400 mg 4-HPR or placebo for 4-6 months with monthly 3-day drug holidays. Following this treatment period, the participant undergoes the planned prophylactic oophorectomy 7-10 days after the first day of her menstrual cycle. The primary objectives are to assess the effect of 4-HPR on ovarian histology; and the effect of 4-HPR on potential surrogate endpoint biomarkers (SEBs): apoptosis (TUNEL and immunohistochemistry of single-stranded DNA), apoptosis regulation (bcl-2 and Bax expression), and one marker of proliferation (MIB-1 protein level). The total duration of the study is three years.

Additional control ovarian tissue will be obtained from: 1) high-risk individuals who are eligible for the trial but uncomfortable waiting 4-6 months for their oophorectomy, and 2) normal, low-risk individuals. These banked tissue samples will assist in evaluating the variability between individuals over time and the significance of SEBs for ovarian cancer. Thus, there will be six group comparisons.

In late June 1999, FCCC received fenretinide and placebo from the NCI. Screening of interested participants has begun. The Family Risk Assessment Program at FCCC has already identified 8 interested women who are planning a prophylactic oophorectomy in an attempt to lower their risk of ovarian cancer. These individuals have been contacted regarding participation, and appointments for enrollment have been scheduled. It is anticipated that the first participant will be enrolled in October 1999.

KEY RESEARCH ACCOMPLISHMENTS

Anticipated key research accomplishments emanating from this research include the following:

- Success in altering the SEBs in this clinical trial format would justify prolonged treatment with fenretinide and provide an alternative to oophorectomy for prophylaxis in women at high risk for ovarian cancer.

- Tissues obtained during this research will be a resource for further studies of molecular carcinogenesis in ovarian cancer. This effort may lead to the identification of specific novel targets for therapy and prevention in patients with hereditary ovarian cancer and the more common sporadic epithelial ovarian cancer.

REPORTABLE OUTCOMES

The research protocol review and approval process was complicated and lengthy. Thus, no individuals have been enrolled to date. However, during this process, data collection and management systems were created in preparation for study activation.

1. Data Entry, Management and Quality Control

The large volume of information to be generated in this project requires the implementation of computer-based tools for the management and coordination of data. The Population Informatics Facility (PIF) is responsible for all database and statistical programming aspects of this study. The purpose of the PIF is to provide Informatics expertise to facilitate the research conducted by investigators at FCCC. PIF personnel designed and developed the appropriate database, created the data entry interface, trained the technicians in its use, and provided regular feedback on data quality.

At recruitment, each subject will be given a unique identification number. Baseline information on health, family and dietary history, along with pretreatment laboratory and clinical test results will be entered onto prepared hardcopy (paper) data collection instruments by a study representative. Upon completion, these forms will be sent to the FCCC Chemoprevention Protocol Office (CPO) where the data will be entered via terminals into the database using the electronic data entry system created by PIF programmers.

At each subsequent follow-up contact, a study representative will complete hardcopy questionnaires containing information on study subject compliance with pill consumption, toxicity symptoms, results of routine blood sample analyses, and clinical observations made by the attending physician. Similarly, the study representative will place results from all laboratory procedures on hardcopy data collection instruments. These forms will be sent to the Protocol Coordinator for data entry. All laboratory records will include the unique identifier and date of collection of the biologic sample.

The information system for this project was built on the system that has been developed by PIF to support the Chemoprevention Clinical Trials at FCCC. As of May 1, 1999, the Chemoprevention Clinical Trials database stores information on 1,526 study subjects from seven chemoprevention trials at FCCC. This DBMS maintains all of the data collected in these studies and is designed to facilitate many aspects of data collection and patient tracking. Based upon the data entered into the database, this software system is capable of performing such tasks as the determination of study eligibility, automated subject randomization and the generation of mailed reminder letters. Most, if not all, of these capabilities have been incorporated into the systems developed for this project.

The existing database management system uses the relational database product ORACLE as the primary software platform for data entry and validation, storage, retrieval, modification, and security. This software system runs on a UNIX-based distributed computing system consisting of 7 DEC Alpha Station RISC computers running under the Digital UNIX operating system. These computers are maintained by the Research Computer Services facility at the Fox Chase Cancer Center. This distributed computing system is an integral part of a Local Area Network (LAN) which provides connections to a Digital VAX computer, IBM compatible PC's, Macintoshes, printers, plotters, and the Internet. The software developed to meet the needs of this study will also use these computing facilities.

On-screen data entry forms, designed to resemble the data collection instruments, will be created using the ORACLE Forms V6.0 software. Data validation will occur both during and after data entry. Range, validity and logical consistency checks will be conducted during the data entry process to ensure data quality. Reports generated from the entered data will be compared to the original data collection instruments to further ensure the accuracy of the data stored on magnetic media. Edits will be conducted using the query-by-form capability of ORACLE. This system of data entry and corrections will allow the data analyst to have access to the most up-to-date and accurate data at any given time. Daily backups of the database will be conducted to protect against accidental corruption or deletion of the data. Statistical computing will be performed using a variety of statistical packages including SAS, BMDP, IMSL, Splus and other custom written programs.

In order to preserve privacy and confidentiality, a series of security measures will be undertaken. Only the person-specific identifier, and date of collection when appropriate, will be stored with study results. Lists of IDs matched with names and addresses will be stored by the investigators in locked filing cabinets. Further, through the use of the security measures available within the operating system (UNIX) and the relational database management software (ORACLE), restrictions will be applied to each user commensurate with their needs to access the data. All new personnel with any access to the data will be trained in the ethics of electronic data access.

2. Case Report Forms

Data from these studies will be kept in a database consisting of 14 data "tables": (1) Initial Contact/On-study; (2) Eligibility Checklist; (3) Health History Data; (4) Baseline Epidemiologic Data (e.g., smoking and alcohol intake, reproductive history, weight, etc.); (5) Concomitant Medications; (6) Diet Data; (7) Pretreatment Signs and Symptoms; (8) Physical Examination; (9) Study Drug Administration; (10) Compliance Measures; (11) Toxicities; (12) Routine Laboratory Studies (e.g., CBC, electrolytes, liver function tests, etc.); (13) Research Studies (Mib-1, apoptosis markers, etc.); and (14) Off-study. Some of these tables will have one record per subject (e.g., Health History Data) while others may have multiple records per subject (e.g., Toxicities), each identified by the individual-specific identification number and date of collection. All tables can be linked by their unique individual identification number (and date of collection, when appropriate).

3. Ovarian Cancer Research Fund, Inc.

Due to significant interest in the prevention of ovarian cancer, the Ovarian Cancer Research Fund, Inc. provided a $50,000 renewable grant for this research. These funds are being used to support expenses associated with this project that are not being funded by the DOD.

CONCLUSIONS

The clinical trial is ongoing; thus, no conclusions can be made at this time.

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APPENDICES - PROJECT I

APPENDIX A
KEY PERSONNEL

Fox Chase Cancer Center

Principal Investigator: Paul F. Engstrom, M.D.
Project Director: Mary B. Daly, M.D., Ph.D.
Co-Investigator: Andrew Godwin, Ph.D.
Co-Investigator: Betsy Bove, Ph.D.
Statistician: Andre' Rogatko, Ph.D.
Project Manager: Carol Cherry, R.N.C., B.S.N., O.C.N.
Genetic Counselor: Josephine Costalas, M.S.
Administrative Assistant: Honey Salador
Data Management: Andrew Balshem
John Malick
Rose Batson
Director of Nursing Research: Andrea Barsevick, R.N., D.N.Sc.

Cooper Hospital/University Medical Center

Network Site Director: Generosa Grana, M.D.
Gynecology Oncology Group: David Warshal, M.D.
James Aikins, M.D.
Thomas Rocereto, M.D.
Risk Evaluation Program Coordinator: Patricia Barse, R.N., M.S.N., A.O.C.N.
Gynecologic Oncology Nurse: Wendy Topeka, R.N., B.S.N., O.C.N.

The Reading Hospital and Medical Center

Network Site Directors: Norman G. Rosenblum, M.D., Ph.D. & Terrance Cescon, M.D.
Cancer Center Program Manager: Patricia Weiser, R.N., C.C.R.A.
Family Risk Assessment Program Coordinator: Marilyn Brennan, R.N., O.C.N.

Bowman Gray Medical Center

Network Site Director: Electra D. Paskett, Ph.D.
Research Associate: Cecilia R. DeGraffinreid, M.H.S., R.R.A.

APPENDIX B

TELEPHONE SCRIPTS

High Risk Women
Ovarian Cancer Patients
Relatives

Ovarian Cancer Registry Script
-High Risk Women-

Hello, this is _____ of Fox Chase Cancer Center's Family Risk Assessment Program (in Philadelphia). Our records indicate you have contacted our program in the past and today I am calling to discuss our ovarian cancer registry project for which you may be eligible. If you are interested I can describe what the study involves and answer any questions you may have.

Is now a good time for us to talk?

Yes _____ Continue

No _____ If no, when can I call you back? _____

Refusal – record reason for refusal below:

_____
_____
_____
_____

Program Description

Let me give you a little bit of information about the registry. The ovarian cancer registry was funded by a grant from the Department of Defense and is being managed by the Family Risk Assessment Program at Fox Chase Cancer Center. Dr. Mary Daly, a medical oncologist, is the director of the program.

The ovarian cancer registry has been established to collect blood and tissue samples, and information from families who have a history of ovarian cancer for the purposes of research. Our aim is to bring together doctors and researchers to learn more about the causes of ovarian cancer and better ways of preventing, detecting, and treating the disease.

If you should agree to participate in the registry we will ask you to do a few things:

1. Complete a questionnaire about your family history of cancer, personal medical history, and lifestyle habits, and agree to complete a follow up questionnaire about your health once a year.
2. Complete a diet questionnaire.
3. Donate 5 tubes of blood (4-5 Tablespoons).
4. If you have had surgery for ovarian cancer, you will be asked to give us permission to contact the hospital where the surgery was performed so we can request a sample of the stored tumor, pathology report, and operative report.
5. If you are the next of kin, you will be asked to give us permission to contact the hospital where [NAME] had her surgery so we can request a sample of the stored tumor, pathology report, and operative report.

Because the donation of a blood sample is for research purposes, you will not receive any personal feedback about the studies conducted on your blood. The samples and information you provide will serve as a resource for researchers. We will keep you abreast of the progress of the research by way of a newsletter. As always, you are free to call us if you wish to know more about the research findings reported in the newsletter or if you have any general questions.

Based upon the information we have discussed, are you interested in participating?

If No – thank the person for his/her time and document below reason for refusal.

_____

_____

_____

If Yes

❑ Would you like to come to FCCC and donate a sample of blood?

❑ Would you like us to send you a blood kit so that you can have your blood drawn at your doctor's office?

Appointment at FCCC

Unfortunately our phlebotomy lab is not open in the evenings. We are able to draw your blood between 7:00 a.m. [Only if someone from the OCCRS staff can be there. Otherwise, make it later in the morning] and 2:30 p.m. On what day and at what time would you like to come?

DAY: _____ TIME: _____

I will call you the day before your appointment to confirm. Thank you for your participation. I look forward to meeting you.

Send Blood Kit

Thank you for your willingness to participate in the registry. In the next week you will receive a package in the mail. It will contain a blood collection kit along with instructions for the technician about how to package and return the blood samples. Additionally you will find a consent form, health history questionnaire and diet questionnaire. Please complete the questionnaires and consent form and return them in the envelope that will be provided for your convenience. Do not include the paperwork with the blood samples. The blood samples must be packaged alone. Fox Chase has provided everything the lab will need to mail the blood samples to us. You will not be responsible for postage.

If you have any questions or concerns please do not hesitate to call me. A toll free number will be included in the package. I'll return your call as quickly as possible. [Repeat name of caller and give phone number.]

Thank you again for your commitment to our research.

Ovarian Cancer Registry Script
-Ovarian Cancer Patients-

Hello, this is _____ of Fox Chase Cancer Center's Family Risk Assessment Program (in Philadelphia). I received your name from [NURSE/DOCTOR] who thought you might be interested in hearing about our ovarian cancer registry project. I am calling to explain the purpose of the registry and how it works, to see if you're interested in participating, and to answer any other questions you may have.

Is now a good time for us to talk?

Yes _____ Continue

No _____ If no, when can I call you back? _____

Refusal – record reason for refusal below:

_____
_____
_____
_____

Program Description

Let me give you a little bit of information about the registry. The ovarian cancer registry was funded by a grant from the Department of Defense and is being managed by the Family Risk Assessment Program at Fox Chase Cancer Center. Dr. Mary Daly, a medical oncologist, is the director of the program.

The ovarian cancer registry has been established to collect blood and tissue samples, and information from families who have a history of ovarian cancer, for the purposes of research. Our aim is to bring together doctors and researchers to learn more about the causes of ovarian cancer and better ways of preventing, detecting, and treating the disease.

If you should agree to participate in the registry we will ask you to do a few things:

1. Complete a questionnaire about your family history of cancer, personal medical history, and lifestyle habits, and agree to complete a follow up questionnaire about your health once a year.
2. Complete a diet questionnaire.
3. Donate 5 tubes of blood (4-5 Tablespoons).
4. If you have had surgery for ovarian cancer, you will be asked to give us permission to contact the hospital where the surgery was performed so we can request a sample of the stored tumor, pathology report, and operative report.
5. Consider talking to some of your family members about the research.

Because the donation of a blood sample is for research purposes, you will not receive any personal feedback about the studies conducted on your blood. The samples and information you provide will serve as a resource for researchers. We will keep you abreast of the progress of the research by way of a newsletter. As always, you are free to call us if you wish to know more about the research findings reported in the newsletter or if you have any general questions.

Based upon the information we have discussed, are you interested in participating?

If No – thank the person for his/her time and document below reason for refusal.

_____

_____

_____

If Yes

We would like to coordinate a meeting with you to review a consent form and obtain your blood sample in conjunction with your upcoming clinic appointment with Dr. [NAME] on [DATE] at [TIME]. Would it be convenient for us to meet at [TIME] in the Outpatient Department registration area?

CONFIRM DAY:_____ TIME:_____

[NOTE TO STAFF: Blood must be drawn before 2:30p.m.]

You will receive a confirmation of the appointment, along with the questionnaires we discussed, in the mail. Thank you for your participation. I look forward to meeting you.

If patient will have blood drawn at her doctor's office requiring blood kit:

Thank you for your willingness to participate in the registry. In the next week you will receive a package in the mail. It will contain a blood collection kit along with instructions for the technician about how to package and return the blood samples. Additionally you will find a consent form, health history questionnaire and diet questionnaire. Please complete the questionnaires and consent form and return them in the envelope that will be provided for your convenience. Do not include the paperwork with the blood samples. The blood samples must be packaged alone. Fox Chase has provided everything the lab will need to mail the blood samples to us. You will not be responsible for postage.

If you have any questions or concerns please do not hesitate to call me. A toll free number will be included in the package. I'll return your call as quickly as possible. [Repeat name of caller and provide phone number].

Thank you again for your commitment to our research.

Ovarian Cancer Registry Script
-Relatives-

Hello, this is _____ of Fox Chase Cancer Center's Family Risk Assessment Program (in Philadelphia). [NAME] gave us permission to contact you about an ovarian cancer registry in which she is participating. She thought that you too would be interested in participating. I am calling to ask if you received a letter telling you of this call, to explain the purpose of the registry and how it works, to see if you're interested in participating, and to answer any other questions you may have.

Is now a good time for us to talk?

Yes _____ Continue

No _____ If no, when can I call you back? _____

Refusal – record reason for refusal below:

_____
_____
_____
_____

Program Description

Let me give you a little bit of information about the registry. The ovarian cancer registry was funded by a grant from the Department of Defense and is being managed by the Family Risk Assessment Program at Fox Chase Cancer Center. Dr. Mary Daly, a medical oncologist, is the director of the program.

The ovarian cancer registry has been established to collect blood and tissue samples, and information from families who have a history of ovarian cancer for the purposes of research. Our aim is to bring together doctors and researchers to learn more about the causes of ovarian cancer and better ways of preventing, detecting, and treating the disease.

If you should agree to participate in the registry we will ask you to do a few things:

1. Complete a questionnaire about your family history of cancer, personal medical history, and lifestyle habits, and agree to complete a follow up questionnaire about your health once a year.
2. Complete a diet questionnaire.
3. Donate 5 tubes of blood (4-5 Tablespoons).
4. If you have had surgery for ovarian cancer, you will be asked to give us permission to contact the hospital where the surgery was performed so we can request a sample of the stored tumor, pathology report, and operative report.
5. If you are the next of kin, you will be asked to give us permission to contact the hospital where [NAME] had her surgery so we can request a sample of the stored tumor, pathology report, and operative report.

Because the donation of a blood sample is for research purposes, you will not receive any personal feedback about the studies conducted on your blood. The samples and information you provide will serve as a resource for researchers. We will keep you abreast of the progress of the research by way of a newsletter. As always, you are free to call us if you wish to know more about the research findings reported in the newsletter or if you have any general questions.

Based upon the information we have discussed, are you interested in participating?

If No – thank the person for his/her time and document below reason for refusal.

_____

_____

_____

If Yes

❑ Would you like to come to FCCC and donate a sample of blood?

❑ Would you like us to send you a blood kit so that you can have your blood drawn at your doctor's office?

Appointment at FCCC

Unfortunately our phlebotomy lab is not open in the evenings. We are able to draw your blood between 7:00 a.m. [Only if someone from the OCCRS staff can be there. Otherwise, make it later in the morning] and 2:30 p.m. On what day and at what time would you like to come?

DAY: _____ TIME: _____

I will call you the day before your appointment to confirm. Thank you for your participation. I look forward to meeting you.

Send Blood Kit

Thank you for your willingness to participate in the registry. In the next week you will receive a package in the mail. It will contain a blood collection kit along with instructions for the technician about how to package and return the blood samples. Additionally you will find a consent form, health history questionnaire and diet questionnaire. Please complete the questionnaires and consent form and return them in the envelope that will be provided for your convenience. Do not include the paperwork with the blood samples. The blood samples must be packaged alone. Fox Chase has provided everything the lab will need to mail the blood samples to us. You will not be responsible for postage.

If you have any questions or concerns please do not hesitate to call me. A toll free number will be included in the package. I'll return your call as quickly as possible. Repeat name of caller and give phone number.

Thank you again for your commitment to our research.

APPENDIX C

TREATMENT QUESTIONNAIRE FOR OVARIAN CANCER PATIENTS

ID#_____
Proxy ID#_____
Date Completed_____

OVARIAN CANCER CONSORTIUM FOR RESEARCH AND SURVEILLANCE TREATMENT QUESTIONNAIRE FOR OVARIAN CANCER PATIENTS

1. How old were you when your ovarian cancer was first diagnosed?

   Age_____

2. At the time this cancer was first diagnosed, was it

   - ❑ only in the ovary or ovaries
   - ❑ spread beyond the ovaries
   - ❑ unknown

Question 3 asks for treatment given for the ovarian cancer at the time it was first diagnosed. This treatment would usually be given within the first year after the original diagnosis. Please do not include treatment given for cancer which came back after the original treatment.

3. Which one of the following treatments did you have for the ovarian cancer at the time it was first diagnosed?

   A. Surgery
   - ❑ no (go to question 3B)
   - ❑ yes
   - ❑ unknown (go to question 3B)

   If **yes**, what type of surgery did you have?
   PLEASE CHECK APPROPRIATE BOX

   - ❑ removal of one or both ovaries
   - ❑ total hysterectomy (removal of uterus, fallopian tubes & ovaries)
   - ❑ other (please specify)

     _____

     _____

B. Chemotherapy

- ❑ no (go to question 3C)
- ❑ yes
- ❑ unknown (go to question 3C)

If **yes**, please check the medications you received and how many cycles of each.

- ❑ Carboplatin _____ cycles
- ❑ Cisplatin _____ cycles
- ❑ Etoposide (VP-16) _____ cycles
- ❑ Taxotere _____ cycles
- ❑ Herceptin _____ cycles
- ❑ Gemcitabine _____ cycles

- Taxol _____ cycles
- Cytoxan _____ cycles
- Ifosfamide (Ifex) _____ cycles
- Topotecan _____ cycles
- Doxil _____ cycles

Other_____
_____
_____

C. Radiation

- ❑ no (go to question 4)
- ❑ yes
- ❑ unknown (go to question 4)

If **yes**, please check the area of the body that was radiated.

- ❑ pelvis
- ❑ whole abdomen
- ❑ radiocative substance (P32) put into abdominal cavity
- ❑ lungs
- ❑ other (please specify) _____
_____
_____

Approximately, how many treatments did you have?_____

D. Other therapies for ovarian cancer

- ❑ no (go to question 5)
- ❑ yes
- ❑ unknown (go to question 5)

If **yes**, please check the type of therapy

- ❑ stem cell transplant
- ❑ Tamoxifen
- ❑ Other (please specify)_____

_____

_____

4. Has the cancer come back (recurred) after the treatments listed above?

- ❑ no
- ❑ yes
- ❑ unknown

If **yes**, please specify any additional treatments_____

_____

_____

_____

_____

_____

_____

THANK YOU FOR TAKING THE TIME
TO COMPLETE THIS QUESTIONNAIRE

APPENDIX D

PARTICIPANT CONSENT FORM

Cooperative Family Registry for Ovarian Cancer Studies
Participant Consent Form

Study Title: Cooperative Family Registry for Ovarian Cancer Studies

Principal Investigator: Mary B. Daly, M.D., Ph.D.
Fox Chase Cancer Center
Family Risk Assessment Program
510 Township Line Road
Cheltenham, PA 19012
(215) 728-2791

Study Purpose: The Fox Chase Cancer Center and other research centers are obtaining blood, tissue and personal data to serve as a resource for research on the causes of ovarian cancer to find new ways of prevention, diagnosis and treatment. Researchers will use blood and tissue samples and personal information from participants to study how genes, lifestyle, and our environment may lead to ovarian cancer. The Registry consists of a bank of information from families with a history of ovarian cancer which will serve as a resource of information and blood and tissue samples for other researchers around the world. It is hoped that someday this research will lead to the prevention of ovarian cancer and in the improvement of its detection, diagnosis and treatment.

Study Participants: I have been invited to participate in this Registry because I fit one of these two categories: 1) a woman diagnosed with ovarian cancer, or 2) a person, aged 18 years or older, with one or more family members diagnosed with ovarian cancer.

Process of Informed Consent: In order for me to decide whether to be part of the Registry, it's important to understand what I am required to do, along with the possible risks and benefits associated with my participation. This process is known as informed consent.

This consent form provides information about the Registry similar to what has been described to me by the Registry staff. The staff will be available to answer any questions I may have now or in the future. Once I have read the consent form and feel I understand the Registry procedures and I decide to participate, I will be asked to sign this form giving my informed consent to participate.

My participation is completely voluntary and I may withdraw at any time.

Study Procedures: Because the primary goal of the Registry is to collect a basic set of information that may help to explain causes of ovarian cancer, each participant will be asked to provide Registry staff with lifestyle, medical and family history information and donate blood and possibly tissue samples. I will be asked to do the following:

1. Complete a questionnaire about my family history of cancer, personal medical history, lifestyle and reproductive information, and my diet. This questionnaire takes approximately 20-30 minutes to complete.

2. Complete a follow-up questionnaire each year to update the Registry files on my health, and to reaffirm my willingness to participate in the Registry. This questionnaire takes approximately 5-10 minutes to complete.

3. Donate five tubes of blood (about 4-5 tablespoons) that will be drawn from a vein in my arm.

4. If I have had a biopsy, or surgery to remove a tumor in the past, sign a form to allow the Registry staff to get copies of my pathology and medical records and to get a portion of my stored tissue.

5. If I am scheduled for a biopsy, or surgery to remove a tumor now or in the future, sign a form to allow the Registry staff to get copies of my pathology and medical reports and to get a portion of the removed tissue.

6. Sign a form to allow the Registry staff to get copies of medical and pathology records, and tissue samples from surgery, that may be available from a deceased relative who had ovarian cancer for whom I may legally allow release.

7. Assist the Registry staff in contacting my family members so the Registry staff can invite them to also participate in the Registry.

Contacting of Family Members: If I am willing to have the Registry staff contact my relatives about participating in the Registry, the Registry staff will ask me to give them my relatives' names, addresses and telephone numbers and the Registry staff will send them a letter of introduction.

The letter of introduction will inform them that they were referred to the Registry by me because of their shared family history of ovarian cancer. The Registry staff will contact them by telephone and ask them to participate in the Registry. They will have the opportunity to decline receiving the telephone call by calling an 800 number. If they do not call to decline the telephone call within two weeks, the Registry staff will call them to describe the Registry and ask if they would like to participate.

If they agree to participate, they will be asked to sign a consent form. No medical or personal history information provided by one member of a family will be discussed with any other family members.

Future Cancer Studies: The personal and family history information and the blood and tissue samples I give the Registry staff are being collected for use in future research. My information and samples will only be released for research purposes to approved researchers.

The exact tests that will be performed in future cancer research studies are not known at this time but are likely to include the following: 1) the study and comparison of pedigrees (family trees); 2) the study of genes and changes in genes that may be involved in hereditary and non-hereditary ovarian cancer development; and 3) the study of how race/ethnicity, age, lifestyle, the environment, and other factors may play a role in ovarian cancer development. At no time will my name and address be given for research purposes without my permission. Instead, requests for use of the information in the Registry will be handled in the following way.

Researchers who are interested in using Registry information must complete a research plan with specific information about their study, including a description of how they will use the Registry information. Plans will be reviewed by a group of experienced scientists who will judge each proposal for its scientific merit, its potential contribution to the prevention or cure of cancer, and for the qualifications of the research team. Because the Registry staff realizes that the information and samples I provide are an invaluable contribution to science, their use in any research study will be carefully weighed.

Each plan will also be judged to make sure that it meets all standards of medical ethics and protects my privacy and confidentiality. If a plan is approved, my information and samples will only be shared by the Registry staff with an approved researcher after my name and my address have been removed and a code number has been given.

If an approved researcher would like to collect more information and/or blood samples, not routinely collected by the Registry staff, I will be contacted to explain the study, and I will have the choice of whether to participate before I am contacted directly.

Research Results: The purpose of this Registry is to compile biologic specimens and personal data to serve as a resource for research on the causes of cancer to find new methods of prevention, detection, and treatment. My samples will serve as that resource. If the research conducted using my samples or anyone else's samples yields information that is of any possible medical benefit, the Registry staff wants me to be the first to have the opportunity to benefit from the new findings. The Registry staff will provide me with that information through a newsletter containing new and interesting discoveries in cancer research, and the progress of the Registry in general. Individual counseling will be available to discuss these research findings and determine if they have any personal medical benefit for me.

Registry Rights: By signing this consent form I agree to give the Registry all rights to the access and control of any obtained blood or tissue. The Registry may retain, preserve, or dispose of these samples and may use them in future research studies for an unlimited period of time.

Sometimes, research on human tissue leads to the discovery of new research products or products used to diagnose or treat disease. The blood and tissue sample that I am providing for the Registry might be used in research studies that may have some commercial application. By signing this consent form, I give up any and all rights I may have in any commercial application

associated with information or samples I have provided as part of my participation in the Registry.

Benefits: Although I will receive no immediate direct benefit from participation in the Registry, the Registry staff hope that the knowledge gained from future research studies, using Registry information will be of benefit to me, my relatives, and future generations.

Potential Risks: The risks of giving blood include the possibility of bleeding and bruising or discomfort. This rarely causes a severe problem.

With any kind of research that involves the study of genes, there are social concerns for me to think about. One possible issue is misuse of information by insurance companies or employers. By not releasing any information about me or my family to my insurance company or employer without my consent, the Registry staff hope to prevent any form of discrimination resulting from my participation in the Registry.

Confidentiality: All personal information and blood and tissue samples obtained for this study will be kept confidential. My information and samples will be given a code number. The list of names and matching code numbers will not be kept with the other study information, and will be available only to the Registry staff.

My questionnaire information will be in a secure place at Fox Chase Cancer Center at 510 Township Line Road, Cheltenham, PA 19012. This information will be added to the Registry computer data files. My blood and tissue samples will be in a secure place at the Ovarian Cancer Research Laboratory at FCCC. The results of future studies conducted may be published or presented to scientific groups, but I will not be identified by name in these publications.

It should be noted that representatives of the U.S. Army Medical Research and Materiel Command are eligible to review research records as a part of their responsibility to protect human subjects in research.

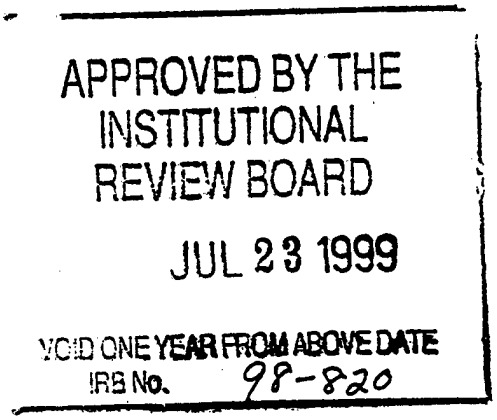
Financial Costs: I will receive no money and I will not be charged for my participation in the Registry. No money will be given by the hospital in the case of a research related injury. Referrals to Clinical Genetics Services can be provided at my request and I may be responsible for the costs of these services.

Withdrawal and Termination: The choice to enter or not to enter this study is mine. I am in a position to make a decision based on my understanding of what the Registry staff has explained about the Registry, as well as what has been described in this form. If I enroll in the Registry, I will still have the right to withdraw at any time. My participation, refusal to participate, or my withdrawal will not affect my or my family's present or future medical care at Fox Chase Cancer Center.

If I wish to withdraw my participation from the Registry at some time in the future, any identifying information stored by the Registry investigators that links me, such as my name and address, to my personal information and blood and tissues samples will be destroyed. Any information or samples I have already given will be retained but will no longer be associated with my name or identification information. Also, I will not be contacted after my withdrawal.

Voluntary Consent: For additional questions concerning this Registry research study or if I am not satisfied with the manner in which this Registry study is being conducted, I may contact the principal researcher, Dr. Mary Daly at (215) 728-2791 or the study project manager, Carol Cherry at (215) 728-3672. Or I may report (without giving my name if I so choose) any complaints to the Institutional Review Board by calling (215) 728-2518, 9:00 a.m. to 5:00 p.m., Monday through Friday, or by addressing a letter to the Institutional Review Board at Fox Chase Cancer Center, 7701 Burholme Ave., Philadelphia, PA 19111. By signing below, I indicate that I have read this form, received acceptable answers to my questions, and have agreed to participate in the Registry, as described above. It is my responsibility to keep the Registry informed of any changes in my address or telephone number. I will receive a copy of this form.

Signature of Participant	Printed Name	Date
Signature of Witness	Printed Name	Date



APPENDIX E

MANUSCRIPT

[CANCER RESEARCH 59, 4973–4983, October 1, 1999]

Expression of OVCA1, a Candidate Tumor Suppressor, Is Reduced in Tumors and Inhibits Growth of Ovarian Cancer Cells[1]

Wendy Bruening,[2] Amanda H. Prowse,[2] David C. Schultz,[2] Marina Holgado-Madruga, Albert Wong, and Andrew K. Godwin[3]

Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111 [W. B., A. H. P., D. C. S., A. K. G.]; Wistar Institute, Philadelphia, Pennsylvania 18014 [D. C. S.]; and Department of Microbiology and Immunology, Jefferson Cancer Institute, Thomas Jefferson University, Philadelphia, Pennsylvania 19107 [M. H-M., A. W.]

ABSTRACT

Loss of all or part of one copy of chromosome 17p is very common in ovarian and breast tumors. *OVCA1* is a candidate tumor suppressor gene mapping to a highly conserved region on chromosome 17p13.3 that shows frequent loss of heterozygosity in breast and ovarian carcinomas. Western blot analysis of extracts prepared from breast and ovarian carcinomas revealed reduced expression of OVCA1 compared with extracts from normal epithelial cells from these tissues. Subcellular localization studies indicate that OVCA1 is localized to punctate bodies scattered throughout the cell but is primarily clustered around the nucleus. Attempts to create cell lines that stably expressed OVCA1 from the cytomegalovirus promoter were generally unsuccessful in a variety of different cell lines. This reduction of colony formation was quantified in the ovarian cancer cell line A2780, where it was demonstrated that cells transfected with plasmids expressing OVCA1 had a 50–60% reduction in colony number as compared with appropriate controls, and only a few of these clones expressed OVCA1, albeit at low levels. The clones that expressed exogenous OVCA1 were found to have dramatically reduced rates of proliferation. Reduced growth rates correlated with an increased proportion of the cells in the G_1 fraction of the cell cycle compared with the parental cell line and decreased levels of cyclin D1. The low levels of cyclin D1 appeared to be caused by an accelerated rate of cyclin D1 degradation. Overexpression of cyclin D1 was able to override OVCA1's suppression of clonal outgrowth. These results suggest that slight alterations in the level of OVCA1, such as would occur after reduction of chromosome 17p13.13 to hemizygosity, may result in cell cycle deregulation and promote tumorigenesis.

INTRODUCTION

Ovarian cancer is the leading cause of death from gynecological malignancy and the fourth leading cause of cancer death among American women, yet little is known about the molecular evolution of ovarian tumors. Only a few candidate tumor suppressor genes in sporadic ovarian cancer have thus far been identified. Although two familial breast/ovarian cancer genes, *BRCA1* and *BRCA2*, have been identified, mutations in sporadic ovarian cancers are rare in these genes. Other recently identified tumor suppressor genes that have been analyzed for mutations in ovarian tumors include *TSG101*, *PTEN*, *DPC4*, and *BARD1*. However, there has been little evidence reported suggesting that these genes are important in the pathogenesis of sporadic ovarian cancers (1–7). In addition, several interesting candidate tumor suppressor genes, *DOC2*, *NOEY2*, and *LOT1*, have recently been identified, and their roles in the development of ovarian cancer are currently being investigated (8–11). The *TP53* tumor suppressor gene is, by far, the most frequently altered gene observed in ovarian cancer. In epithelial ovarian carcinomas, *TP53* mutations are present in ~50% of advanced-stage cancers. However, the low frequency of *TP53* mutations in cancers confined to the ovary and the near absence of mutations in benign and borderline ovarian neoplasms suggest that *TP53* alterations may be a relatively late event in the progression of ovarian cancer (12).

LOH[4] for markers on the short arm of chromosome 17 is one of the most common genetic abnormalities in ovarian cancer. Two regions on 17p13, including *TP53* at 17p13.1 and a more telomeric region at 17p13.3 defined by markers D17S5/30 (equivalent to YNZ22.1) and D17S28 (equivalent to YNH37.3), have received the most attention (13). It has been reported that YNZ22.1 had a rate of LOH as high as 80%, and YNH37.3 showed >65% LOH in ovarian carcinomas. Loss at either D17S5/S30 or D17S28 was observed in 43% of low malignant potential tumors, 80% of carcinomas without metastases, and 90% of advanced-stage carcinomas. Interestingly, in the low malignant potential tumors, allelic losses at YNZ22.1 and YNH37.3 were not accompanied by LOH at *TP53*, suggesting the loss of a more distal tumor suppressor gene in early tumorigenesis (14, 15).

Alterations involving the NYH37.3/YNZ22.1 region on chromosome 17p13.3 are not limited to ovarian cancer. A recent study by the European Breast Cancer Linkage Consortium of 1280 breast tumors found that the frequency of LOH observed on the p arm of chromosome 17 was much higher than that observed on the q arm (16). Up to two-thirds of breast tumors show LOH at the YNZ22.1 locus (17–23), and this finding has been associated with markers of tumor aggression (16, 23–25). Breast tumors with LOH at YNZ22.1 have been associated with a higher risk of recurrence than those showing retention of this region (23, 25). This same region shows frequent LOH in small cell lung cancers (26–28), colon cancers (29), primitive neuroectodermal tumors (30–32), carcinoma of the cervix uteri (33–36), medulloblastoma (37–40), astrocytoma (41, 42), follicular thyroid carcinoma (43), malignant melanoma (44), hepatocellular carcinoma (45), and leukemia and lymphoma (46). In many of these studies, changes on chromosome 17p13.3 occur in the absence of alterations involving *TP53*, suggesting that a tumor suppressor gene(s) residing in this region on chromosome 17p13.3 may be involved in the development of many types of cancers.

We have previously reported the identification of a common region of allelic loss on 17p13.3 in ovarian cancer defined by the markers D17S28 and D17S5/S30 (47). These two loci span <20 kbp (47). By the use of positional cloning strategies, two candidate tumor suppressor genes, *OVCA1* and *OVCA2*, have been identified that map to the region that is most commonly lost in ovarian and breast tumors, chromosome 17p13.3 (47, 48). OVCA1 demonstrates sequence similarity (20% identity) to one of the yeast enzymes in the diphthamide

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[2] The first three authors contributed equally to this work.

[3] To whom requests for reprints should be addressed, at Department of Medical Oncology, Fox Chase Cancer Center, 7701 Burholme Avenue, Room W-304, Philadelphia, PA 19111. Phone: (215) 728-2756; Fax: (215) 728-2741; E-mail: a_godwin@fccc.edu.

[4] The abbreviations used are: LOH, loss of heterozygosity; SSCP, single-strand conformation polymorphism; HA, hemagglutinin; GST, glutathione *S*-transferase; FACS, fluorescence-activated cell sorting; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; VNTR, variable number of tendem repeats; CMV, cytomegalovirus; CD, Cowden disease; LDD, Lhermitte-Duclos disease; BZS, Bennayan-Zonena syndrome.

synthetic pathway, DPH2, and to a number of proteins of unknown function from a variety of organisms, including yeasts, plants, insects, and mammals, indicating that this putative protein family is conserved throughout evolution. However, the amino acid sequence of OVCA1 does not demonstrate any conservation with the sequence of any known functional motif (47, 48). Northern blot analysis revealed that *OVCA1* mRNA expression was lost or dramatically reduced in ovarian tumors and ovarian tumor cell lines (as compared with normal ovarian epithelial cells), indicating that loss or reduction of *OVCA1* expression may contribute to ovarian tumorigenesis (47).

Studies in which genes are expressed in tumor cells have provided proof for the pivotal role of TP53, RB, CDKN2A/p16, and BRCA1 in reverting the transformed phenotype of tumor cells (49–55). Here, we report that *OVCA1* can inhibit proliferation of ovarian tumor cells. In addition, we report the identification of genetic alterations of *OVCA1* in ovarian tumor cell lines and in high-risk breast cancer families. These data strongly suggests that *OVCA1* is a viable candidate for the breast and ovarian tumor suppressor gene at 17p13.3.

MATERIALS AND METHODS

Reagents and Cell Lines. Cell culture reagents were from Life Technologies, Inc. (Gaithersburg, MD); unless otherwise indicated, most other reagents were obtained from Sigma Chemical Co. (St. Louis, MO). Cell lines were obtained from American Type Culture Collection (Manassas, VA) or were derived in our laboratory (HOSE, human ovarian surface epithelial cell lines grown in primary culture; and HIO cell lines, SV40-immortalized human ovarian epithelial cells). A2780 cells were maintained in DMEM supplemented with 10% FCS and 0.2 IU/ml porcine insulin. COS-1, MCF-7, and MDA-MB8 cells were maintained in DMEM supplemented with 10% FCS. T47D cells were maintained in RPMI 1640 supplemented with 10% FCS and 0.2 IU/ml porcine insulin. SKBR3 cells were maintained in McCoy's 5a medium supplemented with 10% FCS. HOSE cells and HIO cell lines were maintained in a 1:1 mixture of medium 199 and MCDB-105 medium, supplemented with 5% FCS and 0.2 IU/ml porcine insulin. Unless otherwise stated, cells were transfected with Superfect (Qiagen, Chatsworth, CA), as described by the manufacturer. The A2780 clones that stably express OVCA1 were obtained after selection in G418 by standard methods and maintained in DMEM supplemented with 10% FCS and 0.5 mg/ml G418.

SSCP Analysis of *OVCA1*. PCR was carried out in a reaction volume of 10 μl containing 100 ng of genomic DNA template, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 1 μM forward and reverse primers, 60 μM dNTPs, 0.1 μCi of [^{32}P]dATP (DuPont-NEN, Boston, MA), 5% DMSO, and 0.5 unit of AmpliTaq DNA polymerase (Perkin Elmer Corp., Foster City, CA). Following an initial denaturation step at 94°C for 4 min, DNA was amplified through 20 cycles consisting of 5 s of denaturing at 94°C, 1 min of annealing at 65°C − 0.5°C/cycle, and 1 min of extension at 72°C. The samples were then subjected to an additional 25 cycles, consisting of 1 min of denaturation at 94°C, 1 min of annealing at 55°C, and 1 min of extension at 72°C and a final extension at 72°C for 5 min. PCR products were diluted 1:10 in 95% formamide, 10 mM NaOH, 0.25% bromphenol blue, and 0.25% xylene cyanol. Diluted products were denatured for 5 min at 95°C and flash-cooled on ice. Four μl were loaded onto a 0.5× MDE gel (AT Biochem, Malvern, PA), prepared according to manufacturer's specifications, and electrophoresed at 6 W for 12–16 h at room temperature in 0.6× TBE [1× TBF, 0.09 M Tris, 0.09 M boric acid, and 0.002 M EDTA (pH 8.0)]. Following electrophoresis, the gel was dried and exposed to autoradiography film at −80°C for 4–24 h. Variant and normal SSCP bands were cut out from the gels after alignment with the autoradiograph, and the DNA was eluted in 100 μl of distilled deoionized H_2O at 37°C for 3 h. Two μl of the eluted DNA were used as template for secondary PCRs, as described above, except that radiolabeled dATP was omitted. Following amplification, the DNA was collected on Wizard resin (Promega, Madison, WI) and eluted in 50 μl of distilled deoionized H_2O, and 50–100 fmol of purified PCR product were subjected to direct sequencing.

Plasmids. The eukaryotic expression vectors pcDNA3 and pcDNA3-*LacZ* were obtained from Invitrogen. The HA antibody tag (YPYDVPDYA) was added to the COOH or NH_2 terminus of the OVCA1 cDNA by standard PCR technology, and the resulting tagged cDNAs were subcloned into pcDNA3 and are referred to as pcDNA3-*HAOVCA1* or pcDNA3-*OVCA1HA*, depending on the location of the HA tag. The plasmid pGFP-C1, which expresses green fluorescent protein, was obtained from Clontech. The cDNA of OVCA1 was fused to the COOH terminus of the green fluorescent protein at the *Bgl*II site to generate the plasmid pGFP-*OVCA1*. To prepare a GST fusion of OVCA1 in bacteria, we subcloned the *OVCA1* cDNA, containing an NH_2-terminal HA tag, into pGEX-2T (Pharmacia).

Production of Anti-OVCA1 Antibodies. The 13-amino acid peptide, RDGPGRGRAPRGC, corresponding to amino acids 20–31 of OVCA1 (where the terminal cysteine was added for conjugation purposes) was synthesized (Research Genetics, Huntsville, AL). Purity of the peptide was confirmed by high-performance liquid chromatography. The peptide was conjugated to malemide activated keyhole limpet hemocyanin (Pierce, Rockford, IL) and used to immunize a New Zealand White rabbit (Cocalico, Reamstown, PA). Two mg of antigenic peptide were covalently linked to Aminolink agarose (Pierce) and used to purify anti-OVCA1 antibody from crude serum by affinity chromatography. The final antibody is referred to as TJ132. The antibody FC21 was produced by immunizing a New Zealand White rabbit (Cocalico) with a bacterially expressed carboxyl terminal portion of OVCA1 (amino acids 330–443). The resulting antiserum was immunoaffinity purified on Aminolink agarose covalently linked to bacterially expressed GST-OVCA1.

Purification of Bacterially Expressed OVCA1. BL21 bacteria were transformed with pGEX2T-*OVCA1*. Expression of the fusion protein was induced with 1 mM isopropyl-β-thio-galactopyranoside (Stratagene, La Jolla, CA). The bacteria were lysed by sonication, and GST-OVCA1 was purified from the soluble fraction by binding to glutathione-Sepharose 4B (Pharmacia). Pure OVCA1 was released by digesting with thrombin (Pharmacia), or the GST-OVCA1 fusion was eluted with excess glutathione. PET-*OVCA1* (nucleotides 1011–1350) was expressed in BL21 bacteria and purified as an insoluble inclusion body by repeated washing of the insoluble fraction with 1% Triton X-100. The insoluble pellet was solubilized in 8 M urea-2% SDS. The protein (OVCA1 amino acids 330–443) was further purified by SDS-PAGE. The gel slice containing the protein was homogenized and used to immunize rabbits.

Preparation of Protein Extracts from Human Tumor Specimens. Normal human tissues were obtained from Clontech. Tumors were snap-frozen after surgical removal and stored in liquid nitrogen until use. One g of tumor tissue was rinsed twice with cold PBS and minced finely into small pieces. Tissue pieces were suspended in 1 ml of PBSTDS [0.137 M NaCl, 2.68 mM KCl, 10.6 mM Na_2HPO_4, 1.47 mM $K_2H_2PO_4$, 1% (v/v) Triton X-100, 0.5% (w/v) deoxycholate, 0.1% (v/v) SDS, 0.004% (w/v) NaF, 100 mg/ml phenylmethylsulfonyl fluoride, 1 mg/ml aprotinin, 1 mg/ml leupeptin, and 2 mM sodium orthovandate, (pH 7.4)] and ground with a Polytron tissue grinder at 300–400 rpm for two 30-s intervals at 4°C. Tissue homogenates were clarified by centrifugation at 100,000 × *g* for 1 hour at 4°C. Lipid layers were removed, and cytosolic extracts were aliquoted, snap-frozen in liquid nitrogen, and stored at −80°C. Quantitation of protein was achieved using a bicinchoninic acid/copper (II) sulfate assay (Sigma).

SDS-PAGE and Western Blot Analysis. Fifty μg of total protein extract from tissues or 20 μg of total protein from cell extracts, unless otherwise stated, were separated by standard SDS-PAGE and transferred to Immobilon-P (polyvinylidene difluoride; Millipore, Bedford, MA). The membranes were blocked with 3% BSA and probed with the anti-OVCA1 antibody TJ132, or blocked with 3% dried milk and probed with the indicated antibody. The signal was visualized using anti-rabbit antibodies coupled to HRP (Amersham) and developed using ECL reagents, as recommended by the manufacturer (Amersham).

Subcellular Fractionation. HOSE cells were homogenized in ice-cold hypotonic homogenization buffer [40 mM Tris (pH 7.4), 1 mM EDTA, 1 mM EGTA, 1 mM DTT, and 10% glycerol]. The nuclei were pelleted by centrifugation at 2500 rpm for 10 min. The supernatant was collected, and insoluble debris was pelleted at 180,000 × *g* for 30 min to give the cytosol fraction. The nuclear pellet was washed twice with homogenization buffer containing 0.1 M KCl. The nuclear pellet was then extracted with homogenization buffer plus 0.45 M KCl for 1 h on ice, with frequent vortexing. Insoluble debris was pelleted at 180,000 × *g* for 30 min to obtain the nuclear fraction.

Immunofluorescent Staining and Imaging. COS-1 cells were transfected with the indicated plasmids using Lipofectamine (Life Technologies, Inc.) or Superfect (Qiagen), as directed by the manufacturer. Forty-eight h after trans-

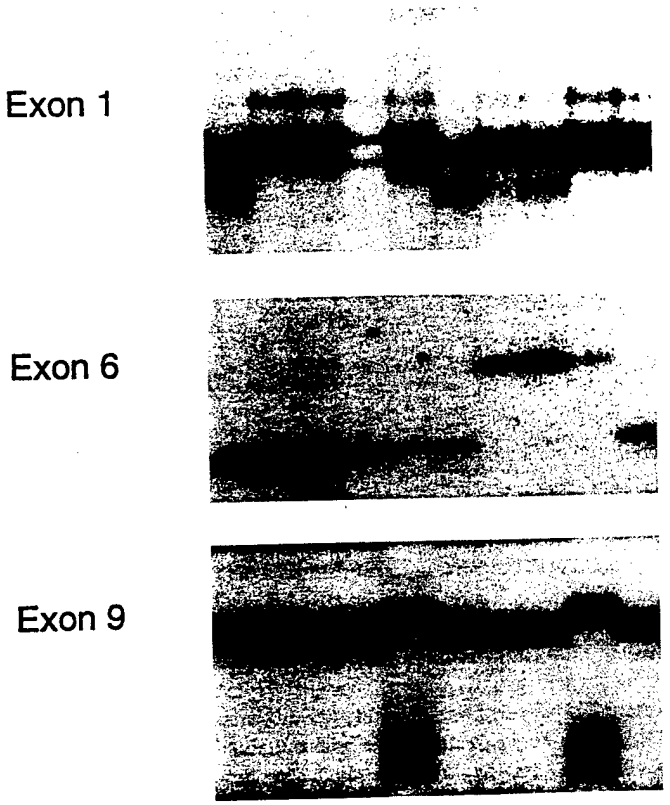

Fig. 1. Mutational analysis of OVCA1. Each exon and its surrounding intronic regions was amplified by PCR and analyzed for sequence variants by SSCP, as described in "Materials and Methods." Examples of the SSCP gel patterns are shown for 10 tumor samples for exons 1, 6, and 9. The exon 1 region has several sequence variants, shown in *Lanes 1, 5, 6, 7,* and *8 (left to right)*; exon 6 has one sequence variant, shown in *Lanes 7, 8,* and *9*; and exon 9 has one sequence variant, shown in *Lanes 5* and *9*. All variants seen by SSCP were verified by directly sequencing a separate PCR.

fection, the cells were visualized. For green fluorescent proteins, the living cells were observed with a Nikon TE 800 upright microscope. For visualizing HA-tagged OVCA1 proteins, the cells were fixed for 20 min in 3.7% formaldehyde-PBS and then for 30 s in methanol. The cells were then stained with an anti-HA rabbit polyclonal antibody Y-11 (Santa Cruz Biotechnology, Santa Cruz, CA) in TBS [50 mM Tris (pH 8.0) and 140 mM NaCl] plus 0.03% Triton X-100 and 10% FCS. The staining was visualized with an antirabbit antibody coupled to Texas Red (Jackson Immunoresearch Laboratory, Inc.). The stained cells were observed on a Biorad MRC 600 laser scanning confocal microscope, using COMOS Version 7.0.1 software. The images were rendered and pseudocolored with Voxel View 2.5.1 (Vital Images) software. Final prints were made using a codonics dye sublimation printer.

Stable Colony Formation Assay. A2780 cells (2.5×10^5 per 60-cm plate) were cotransfected with 5 μg of pcDNA3-*LacZ* and 2.5 pmol of pcDNA3 control vector, pcDNA3-*HAOVCA1*, or p53 expression plasmid or the cyclin D1 expression plasmid. At 24 h posttransfection, G418 (Life Technologies, Inc.) was added to a final concentration of 0.5 mg/ml, or cells were stained for transient β-galactosidase activity. Antibiotic selection was continued for 10–14 days. Colonies were fixed with 0.2% formaldehyde and stained with 0.2% (w/v) crystal violet, and colonies containing >50 cells were scored.

Growth Curve Analysis. Cells were removed from the flask by trypsinization. The trypsin was inactivated by addition of complete medium to a final volume of 10 ml. One hundred μl of cell suspension were diluted in 20 ml of Isoton solution (Coulter, Miami, FL), and the number of cells quantified on a Z1 Coulter counter (Coulter). Cells (200,000) were plated in triplicate in 35-mm tissue culture dishes and incubated at 37°C and 5.0% CO_2. Cells were counted in 24-h intervals by trypsinization and resuspension of cells in 10 ml of Isoton (Coulter) and counted on the Z1 Coulter counter (Coulter).

Pulse-Chase Labeling. Cells were seeded into 60-mm dishes and grown until they were 60% confluent. They were starved in minus-methionine medium (ICN) for 30 min, and then Trans35-Label (ICN) was added to 500 μCi/ml and the cells were labeled for 30 min. The radioactive medium was removed, and the cells were washed with large volumes of complete medium and then incubated in complete medium for the indicated times. The cells were then lysed in 100 μl of PBSTDS. Insoluble debris was pelleted, and the lysates were diluted 10-fold into RIPA buffer [10 mM Tris (pH 8.0), 150 mM NaCl, 1% NP40, 0.1% SDS, and 0.5% deoxycholate]. Anti-cyclin D1 antibody (Santa Cruz Biotechnology) was added and the immunoprecipitates were collected on Protein A beads (Life Technologies, Inc.) and washed well with RIPA buffer. The immunoprecipitates were released by boiling in SDS-PAGE loading buffer and were separated by 12% SDS-PAGE. The amount of label incorporated into cyclin D1 was quantitated by Phosphoimager (Fuji).

FACS Analysis of Stable Transfectants. Cells (500,000) were seeded in 10 ml of complete medium supplemented with 0.5 mg/ml G418. Seventy-two h postseeding, cells were harvested and 1 million cells were prepared for FACS analysis by resuspending cell pellets in 1 ml of staining buffer [3.4 mM sodium citrate, 10 mM NaCl, 0.1% (v/v) NP40, and 75 mM ethidium bromide] and stored at 4°C for no more than 3 days. The cells were filtered through a 37-μm nylon mesh and then analyzed by a flow cytometer (Becton Dickinson, San Jose, CA). Data for 20,000 events were gathered by CellQuest (Becton Dickinson, San Jose, CA) and analyzed by MacCycle (Phoenix Flow Systems, San Diego, CA).

TUNEL Staining. Cells were plated on coverslips and stained for TUNEL using an *in situ* cell death detection kit, as recommended by the manufacturer (Boehringer Mannheim).

RESULTS

Mutational Analysis of *OVCA1* by SSCP. SSCP analysis was conducted on 50 ovarian tumors independent of LOH status for markers on 17p13.3 and on 20 breast tumors demonstrating allelic loss of *OVCA1* and retention of *TP53*. Multiple sequence variants were identified throughout the gene (Fig. 1; Table 1). These sequence variants were deemed to be polymorphisms because these same alterations were either found in the corresponding germ line or resulted in either conservative or silent amino acid substitutions. The frequency of these putative polymorphisms was determined by SSCP analysis of 100 chromosomes from control individuals (Table 1). In addition, we identified two nonconservative amino acid substitutions: alanine 34 changed to an aspartic acid residue and serine 389 changed to an arginine residue. Each alteration was detected in the germ line of a woman with early-onset breast cancer who reported a family history of the disease. In both cases, the missense mutation/rare polymorphism was retained in the corresponding breast tumor DNA and showed reduction to homozygosity (data not shown). Evaluation of >100 control chromosomes has failed to detect these sequence variants. The individual carrying the A34D missense variant was diagnosed with breast cancer at age 37 and reported a history of one

Table 1 *Nucleotide sequence variants observed in* OVCA1 *in tumors*[a]

Exon	Codon	Base	Change	Result	Frequency[b]
1	7	2	C→T	Ala→Val	0.39
2	34	2	C→A	Ala→Asp	0.00
2	72	3	C→T	Ala→Ala	ND
4	104	3	G→A	Val→Val	ND
4	138	3	G→T	Leu→Leu	ND
5	188	3	G→A	Ser→Ser	0.20
9	335	1	C→G	Leu→Val	0.09
9	337	3	C→T	Pro→Pro	0.18
11	389	3	C→A	Ser→Arg	0.00
12	432	3	C→T	Ser→Ser	0.01
13	NC		C→G		ND

[a] The variants shown are those that were detected in the coding and 3′ untranslated region regions. Sequence variants that were detected in the promoter and in introns 5, 6, 11, and 12 are not listed. Codon refers to the amino acid affected by the nucleotide change. Base indicates the nucleotide position of the codon affected. Change describes the nature of the nucleotide alteration. Result describes the affect the nucleotide alteration has on the amino acid. ND, not determined; NC, noncoding sequence.

[b] Allele frequency in control population was determined by examination of 100 chromosomes from unaffected individuals.

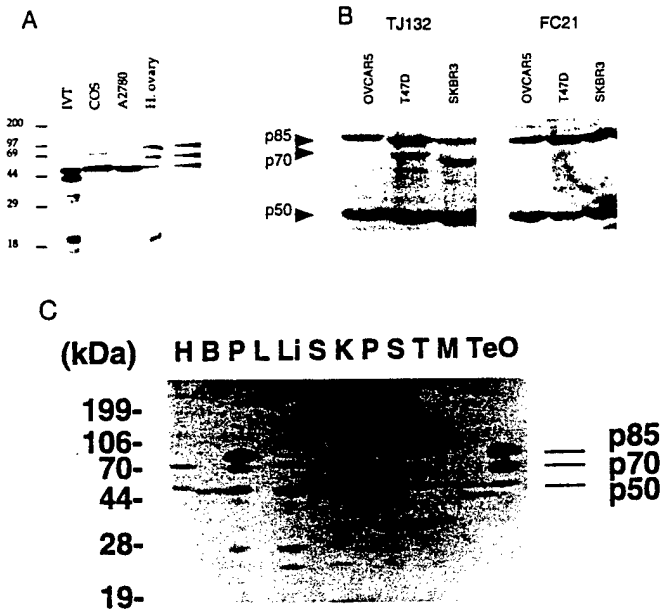

Fig. 2. Characterization of OVCA1 expression. *A*, extracts from the indicated tissues and cell lines were separated by 10% SDS-PAGE and transferred to Immobilon-P, as described in "Materials and Methods." The blot was then probed with the anti-OVCA1 antibody TJ132, as described in "Materials and Methods." *Lane IVT, in vitro* translated pcDNA3-*HAOVCA1*; *Lane COS*, extract of COS-1 cells that had been transfected with pcDNA3-HAOVCA1; *Lane A2780*, extract of the ovarian tumor cell line A2780: *Lane H. ovary*, extract of whole human ovary. *Arrowheads*, three different polypeptides that TJ132 recognizes (p50, p70, and p85). *B*, 20 μg of each indicated cell line extract were separated in duplicate by 10% PAGE, transferred to Immobilon-P, and probed with the indicated antibodies, as described in "Materials and Methods." One of the duplicate blots was probed with the anti-OVCA1 antibody TJ132, and the other was probed in parallel with the anti-OVCA1 antibody FC21. OVCAR-5 is a cell line derived from an ovarian tumor, whereas T47D and SKBR3 are cell lines derived from breast tumors. *C*, 50 μg of extracts from various human tissues (Clontech) were separated by 12% SDS-PAGE and processed for Western blotting, as described in "Materials and Methods." The blot was probed with the anti-OVCA1 antibody TJ132. *Lane H*, heart; *Lane B*, brain; *Lane P*, placenta; *Lane L*, lung; *Lane Li*, liver; *Lane S*, skeletal muscle; *Lane K*, kidney; *Second Lane P*, pancreas; *Second Lane S*, spleen; *Lane T*, thymus; *Lane M*, mammary gland; *Lane Te*, testis; *Lane O*, ovary.

first-degree relative and two second-degree relatives with the disease (ages of onset unknown). The individual carrying the S389R missense variant was diagnosed with breast cancer at age 49. She reported that her mother was affected with breast cancer at age 55 and that two maternal aunts were diagnosed with the disease at 61 and 65 years of age. The functional significance of these mutations is not yet clear; preliminary experiments exploring their effect on the OVCA1 protein are presented below.

Southern Blot Analysis of OVCA1. To assess deletions or rearrangements within the OVCA1 gene, we performed Southern blotting of 60 normal/ovarian tumor DNA pairs using the full-length *OVCA1* cDNA as the probe. The vast majority of the tumors had lost one copy of the *OVCA1* gene. No rearrangements or large interstitial deletions were detected in the remaining copy. However, a 7-kbp *Eco*RI fragment was observed to be variable in length due to changes in the VNTR, *i.e.*, YNH37.3, which is intragenic to *OVCA1* (data not shown). We did not observe any correlation between the length of the VNTR fragment and an increased risk of developing ovarian cancer.

Western Blot Analysis of OVCA1. Conceptual translation of *OVCA1* predicts a 443-amino acid protein with M_r ~50.000. An antibody that recognizes 11 amino acids at the NH_2 terminus of OVCA1 was prepared by immunizing rabbits with a peptide. The antiserum was affinity-purified and was designated TJ132. Another antibody that recognizes the COOH terminus of OVCA1 (amino acids 330–443) was prepared by immunoaffinity purification following immunization of rabbits with a bacterially expressed polypeptide and was designated FC21. Both antibodies were able to recognize bacterially expressed OVCA1 by Western blotting (data not shown). In addition, these antibodies were able to recognize a protein of M_r ~50,000 in extracts prepared from COS-1 cells that had been transiently transfected with pcDNA3-*HAOVCA1* and in whole-cell lysates from the ovarian tumor cell line A2780 (Fig. 2*A*). Recognition of this M_r 50,000 protein could be competed with a molar excess of the antigenic peptide, indicating that the antibodies recognize the authentic OVCA1 protein (data not shown). In addition to the M_r 50,000 protein, both antibodies detected proteins of M_r ~85,000, as observed in extracts prepared from a variety of sources, including normal human tissues, primary cultures of HOSE cells and a number of cell lines (Figs. 2 and 3; data not shown). The NH_2-terminal antibody TJ132 also recognized proteins of M_r ~70,000, but these species were variable in amount and presence and were not recognized by antibodies directed against the COOH terminus of the protein. The secondary antibody alone did not recognize any of the three proteins (M_r 50,000, 70,000, and 85,000; data not shown). The identity of the M_r 70,000 and M_r 85,000 proteins is unknown, as are their relationships with the M_r 50,000 OVCA1 protein; however, the available evidence suggests that the M_r 85,000 form is an alternatively spliced or posttranslationally modified form of OVCA1 and that the p70 form is either unrelated to OVCA1 or is a breakdown product of the p85 form. Alternatively, the p85/p70 forms could be unrelated, cross-reacting proteins. However, this is unlikely because completely different anti-OVCA1 antibodies recognize the p85 protein, and recognition of the M_r 85,000 protein by TJ132 can be competed with a molar excess of

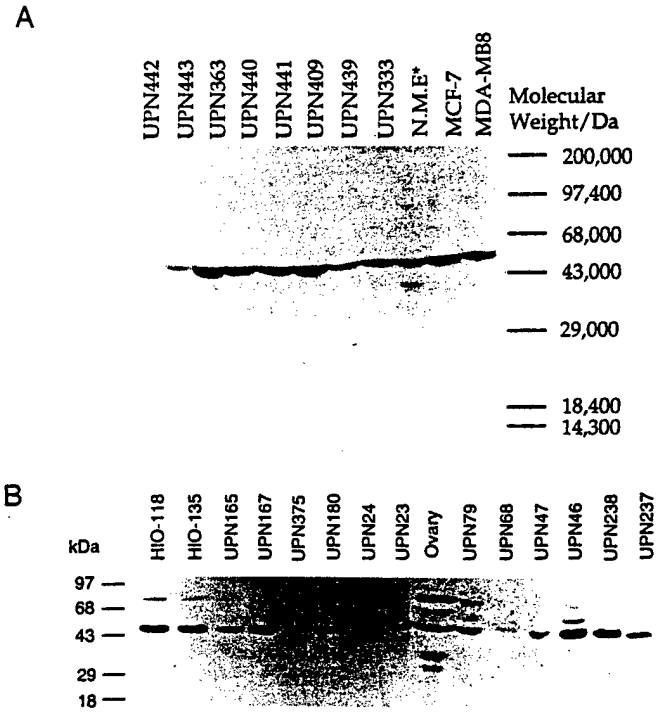

Fig. 3. Expression of OVCA1 in tumors. *A*, 50 μg of each sample were separated by 12% SDS-PAGE and transferred to Immobilon-P, as described in "Materials and Methods." The blot was probed with the anti-OVCA1 antibody TJ132. *UPN*, extracts from primary tumors; *MCF-7* and *MDA-MB8*, extracts from cell lines derived from breast tumors; *N.M.E**, extract from normal breast epithelial cells grown in short-term primary tissue culture. Equal loading of the blots was confirmed by staining with Coomassie Blue for total protein after probing. *B*, protein extracts (50 μg) from primary ovarian tumors (*UPN*), normal human ovarian surface epithelial cell lines (*HIO*), and a normal ovary (*Ovary*) were separated by 12% SDS-PAGE and transferred to Immobilon-P, as described in "Materials and Methods." The blot was probed with the anti-OVCA1 antibody TJ132. Equal loading of the blots was confirmed by staining the blots for total protein with Coomassie Blue after probing.

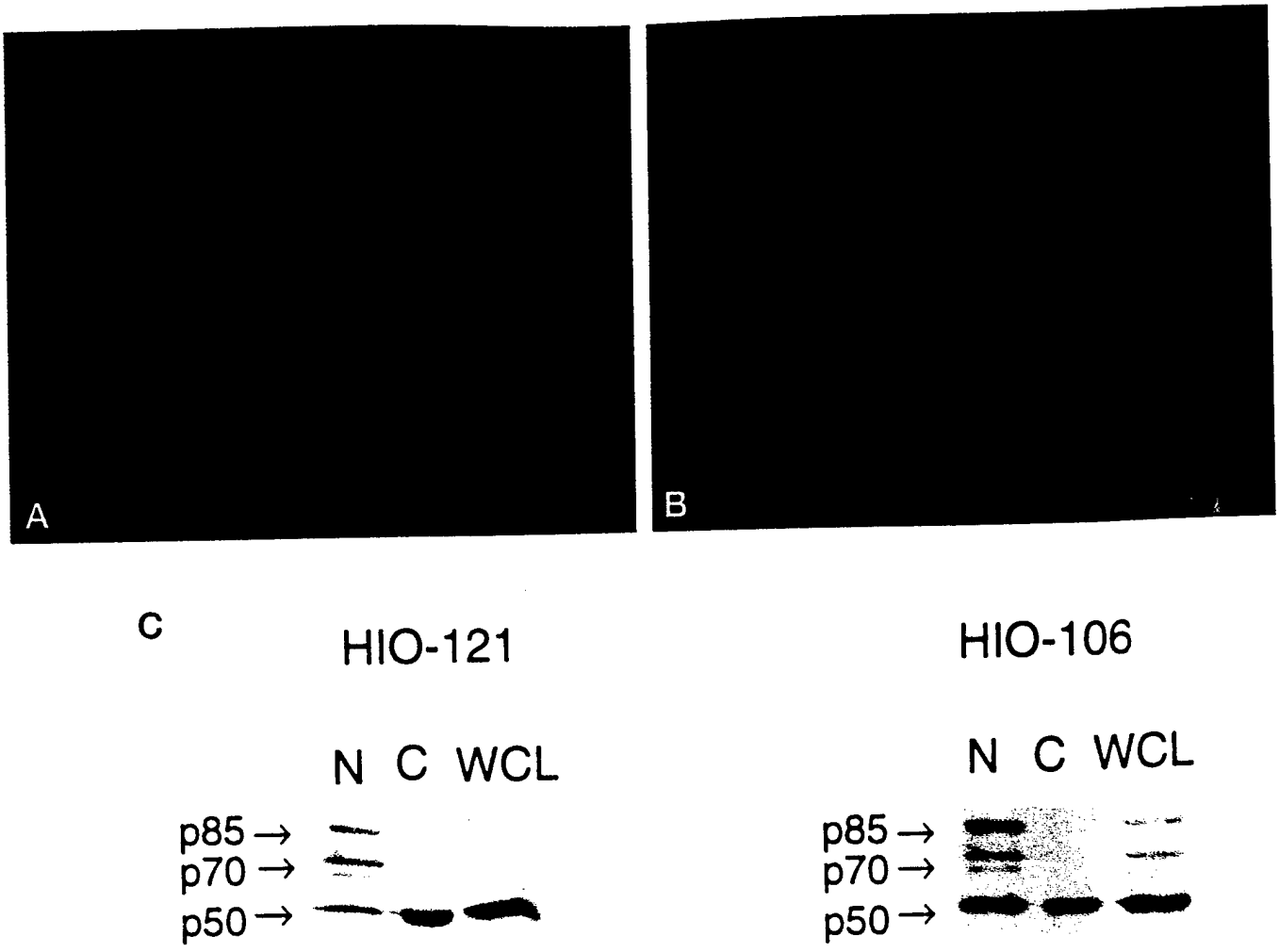

Fig. 4. Subcellular localization of OVCA1. COS-1 cells were transiently transfected with pcDNA3 or with pcDNA3-*OVCA1HA*. Forty-eight h after transfection, the cells were fixed and stained with an anti-HA tag antibody (Y-11; Santa Cruz Biotechnology), as described in "Materials and Methods." *A*, cells transfected with pcDNA3; *B*, cells transfected with pcDNA3-*OVCA1HA*. At the level of sensitivity needed to obtain high resolution of the OVCA1HA staining, the mock-transfected cells are not visible. *C*, subcellular fractionation of SV40-immortalized human ovarian surface epithelial cell lines (*HIO*). Cell lines were fractionated as described in "Materials and Methods," and 50 μg of the corresponding extracts were separated by 12% SDS-PAGE and transferred to Immobilon-P. The Western blot shown was probed with the anti-OVCA1 antibody TJ132. *Lane WCL*, whole-cell extract; *Lane C*, cytoplasmic fraction; *Lane N*, nuclear fraction.

the antigenic peptide. Because FC21 and TJ132 gave almost identical patterns by Western blotting (Fig. 2*B*), most of the data shown used only the antibody TJ132.

OVCA1 was found to be expressed in many different tissues (Fig. 2*C*). In some cases, the M_r 70,000 and M_r 85,000 proteins were very prominent, whereas the M_r 50,000 protein was less so (notably the ovary and placenta) and, in other tissues, the p50 form was predominant (liver and thymus). Note that, although extracts from total breast tissue appeared to express little or no OVCA1 (Fig. 2*C*), breast epithelial cells did express the p50 OVCA1 protein (Fig. 3*A*, *N.M.E.**). We explain this apparent discrepancy as being due to epithelial cells making up only a low percentage of the total breast. Analysis of breast and ovarian tumor extracts demonstrated variable expression levels of p50 and an almost complete absence of the p70/p85 species (Fig. 3). Expression levels of p50 were reduced as compared to normal epithelial cells in 21 of 59 ovarian (37%) and 18 of 46 breast (39%) carcinomas. p85 and p70 were not detected in the majority of tumors analyzed (100% of breast tumors and 85% of ovarian tumors) (Fig. 3). No correlation was evident between reduced expression and clinical prognostic factors.

Subcellular Localization of OVCA1. To aid in understanding the function of OVCA1, we determined its subcellular localization. COS-1 cells were transfected with either an empty vector pcDNA3 or with pcDNA3-*OVCA1HA*, which expresses OVCA1 fused to a COOH-terminal HA tag. Immunostaining of transfected cells with an anti-HA antibody (Y-11; Santa Cruz Biotechnology) indicates that OVCA1 is located throughout the cell. A widespread diffuse staining was seen, in addition to strongly staining punctate bodies (Fig. 4 *A* and *B*). These bodies were scattered throughout the cell and were heavily clustered around the nucleus. A similar pattern was obtained in immortalized HOSE cells transfected with pcDNA3-*OVCA1HA* and when the cells were immunostained with the specific anti-OVCA1 antibody, TJ132 (data not shown). To further confirm the localization, we fused OVCA1 to the COOH terminus of the green fluorescent protein. COS-1 cells expressing the green fluorescent protein-OVCA1 fusion again demonstrated a punctate, primarily perinuclear localization of the protein set against a weaker, diffuse staining throughout the cell (data not shown).

Fractionation studies confirmed that the M_r 50,000 OVCA1 protein is located throughout the cell (Fig. 4*C*). However, the M_r 70,000 and M_r 85,000 species appeared to be exclusively located within the nucleus (Fig. 4*C*).

Suppression of Clonal Outgrowth. Attempts to generate cell lines that stably expressed OVCA1 were unsuccessful. Very few clones

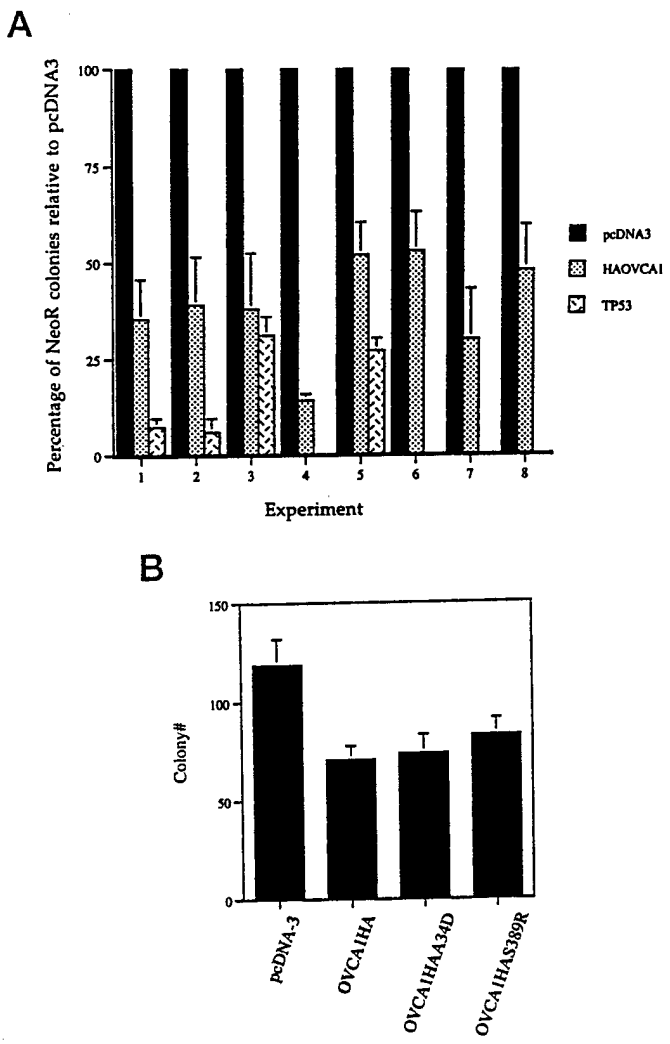

Fig. 5. Suppression of clonal outgrowth by OVCA1 in A2780 ovarian cancer cells. A2780 cells were transfected with the indicated plasmids and selected for resistance to G418, as described in "Materials and Methods." Ten to 14 days posttransfection, the colonies were stained and counted. *A*, the percentage of G418 (Neo^R) colonies that formed relative to the number formed after transfection with pcDNA3 (defined as 100% in each experiment) along the *ordinate*. *Abscissa*, data from eight independent transfection experiments. A wild-type *TP53* expression vector was included in some experiments as a positive control for colony suppression. *B*, the total number of colonies obtained after transfection with the indicated plasmids and selection with G418. pcDNA3 is the parent vector; OVCA1HA expresses the wild-type OVCA1 plus a COOH-terminal HA tag; and A34D and S389R refer to the point mutations, discussed in the text, that were introduced into the wild-type OVCA1HA construct using standard PCR technology. *Columns*, means of three independently repeated experiments; *bars*, SD.

were found to express OVCA1, and those that did expressed only low levels of the protein. This phenomenon was consistently observed in a number of different cell types (RAT-1, U2OS, MCF-7, HIO118, and T47D cells; data not shown). To quantitate this effect, we transfected equimolar amounts of a mammalian expression vector containing the NH_2-terminal HA-tagged OVCA1 open reading frame (pcDNA3-*HAOVCA1*) and an empty expression vector (pcDNA3) into the ovarian cancer cell line A2780. A2780 cells were chosen for further analysis because they are well-characterized ovarian tumor cells that normally express fairly low levels of OVCA1 p50 and almost no p85/p70 OVCA1 (Fig. 2). As a positive control for growth suppression, an expression vector that expresses wild-type p53 protein was included in some of the colony number experiments. Evaluation of colony formation in the presence of geneticin (G418) consistently resulted in a 50–60% reduction in colony number in cells transfected with the *OVCA1* expression construct compared with controls (Fig. 5 *A*). This effect was reproducibly observed in more than four independent transfection experiments. Suppression of clonal outgrowth was independent of plasmid DNA purity (we tested three different preparations of plasmid DNA) whether equivalent molar amounts or microgram amounts of plasmid were transfected. Furthermore, experiments in which an expression vector containing the gene encoding for the β-galactosidase protein were cotransfected with *OVCA1* indicate that the reduction in clonal outgrowth is not an artifact due to differences in transfection efficiency (data not shown).

The A34D and S389R alterations described above, detected in the germ line of women with breast cancer and with a strong family history of the disease, were rebuilt into the pcDNA3-*OVCA1HA* expression plasmid using standard PCR technology. Both altered proteins were expressed well in transient transfection assays (data not shown). However, both alterations were found to suppress colony formation 50–60%, as compared with controls, similar to wild-type OVCA1 (Fig. 5*B*).

Growth Kinetics of Stable Transfectants. To verify that the suppression effect was due to exogenous OVCA1 expression, individual colonies were clonally expanded after G418 selection. A total of seven colonies from pcDNA3 vector control transfected cells and 15 colonies from pcDNA3-*HAOVCA1* transfected cells were amplified following selection in G418 for 10 days. All colonies selected from pcDNA3 vector control plates expanded and formed stable cultures. In contrast, only 9 of 15 colonies selected from pcDNA3-*HAOVCA1* transfected cells expanded to form a stable culture. Because an in-frame HA epitope was fused to the open reading frame of OVCA1 at the NH_2 terminus, the level of exogenous protein produced in these clones could be monitored. Western blot analysis revealed that there was approximately equimolar expression of exogenous and endogenous OVCA1 in only four of nine stable pcDNA3-*HAOVCA1* clones (OV-5, OV-6, OV-9, and OV-13; Fig. 6).

Of the HAOVCA1 transfectants with exogenous expression, no major differences in morphological features were observed when compared to parental A2780 cells (data not shown). Cells were plated at limited dilutions and monitored for growth kinetics. Two independent clones, OV-5 and OV-13, displayed ~8- and 10-fold reductions in total cell number compared with expression vector controls and parental A2780 cells, respectively. A third clonal line, OV-9, demon-

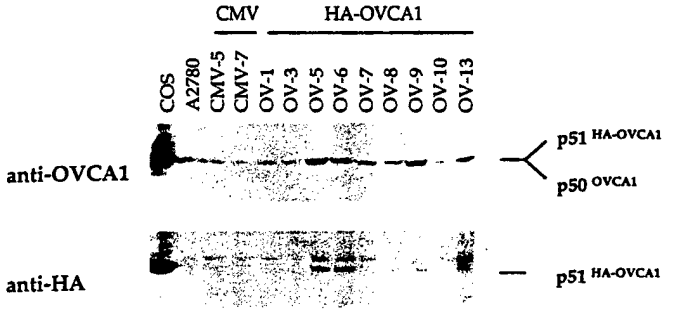

Fig. 6. Maintenance of exogenous OVCA1 expression in stable transfectants of A2780 ovarian cancer cells. A2780 cells were transfected with an *HAOVCA1* expression vector and then selected for resistance to G418, as described in "Materials and Methods." Clones were chosen at random and amplified. After amplification, extracts were prepared from the cells, as described in "Materials and Methods." Ten μg of each extract were separated by duplicate 10% SDS-PAGE. The gels were transferred to Immobilon-P and probed with the indicated antibodies, as described in "Materials and Methods." *Top*, total OVCA1 antigen was detected with the anti-OVCA1 antibody TJ132. *Bottom*, exogenous OVCA1 antigen was detected with the anti-HA mAB 16B12 (BabCo, Richmond, CA). *Lane COS*, protein extract prepared from COS cells transiently transfected with an HAOVCA1 expression vector; *Lanes A2780*, protein extract prepared from the parental cell line; *Lanes CMV*, cell lines derived from pcDNA3 transfected cells; *Lanes OV*, cell lines derived from *HAOVCA1*-transfected cells.

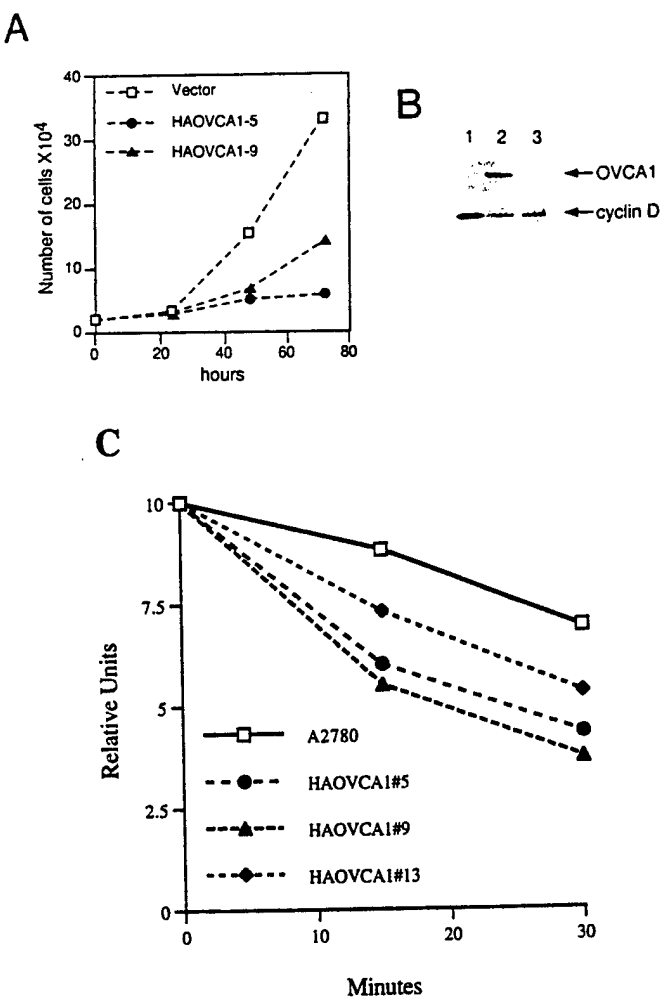

Fig. 7. Suppression of growth rate by OVCA1. *A*, the proliferation of A2780 clonal lines was monitored over time, as described in "Materials and Methods." Vector (□; CMV-5) is A2780 cells that have stably integrated the plasmid pcDNA3; HAOVCA1-5 (●; OV-5) and HAOVCA1-9 (▲; OV-9) are two lines that stably express OVCA1. The parental cell line, A2780, gave results that were virtually identical to those of the CMV-5 cell line (data not shown), and the stably expressing OVCA1 clone OV-13 gave results similar to that of OV-5 (data not shown). The graph represents the growth rates for the cell lines over the indicated time period and is representative of a number of independent experiments. *Ordinate*, number of cells (10^4); *abscissa*, days in 24-h time points. *B*, OVCA1 expression (Western blot probed with the anti-HA tag antibody Y-11; Santa Cruz Biotechnology) and cyclin D1 expression (the Western blot showing OVCA1 expression was reprobed with an anti-cyclin D1 antibody; Santa Cruz Biotechnology) in the indicated cell lines: *Lane 1*, CMV-5; *Lane 2*, OV-5; *Lane 3*, OV-9. The parental cell line, A2780, gave results that were virtually identical to those of the CMV-5 cell line (data not shown), and the stably expressing OVCA1 clone OV-13 gave results that were similar to those of OV-5 (data not shown). *C*, the stability of cyclin D1 was monitored by pulse-chase [^{35}S]methionine labeling of the indicated cells followed by immunoprecipitation of the labeled cyclin D1. The immunoprecipitates were separated on SDS-PAGE and quantitated by Phosphoimager (Fuji). *Abscissa*, minutes after initiation of the chase; *ordinate*, relative units of labeling incorporated into cyclin D1. The graph is representative of a number of independent experiments.

strated a 4-fold reduction in total cell number over the same time interval compared with controls (Fig. 7*A*). On the basis of these growth curves, the cell doubling times between parental A2780 and OVCA1-expressing stable clones were found to be considerably different. A2780 cells doubled 2–2.5 times during a 24-h period, whereas OV-5, OV-9, and OV-13 doubled ~1–1.5 times during the same time interval. Consistent with the reduced growth rate, the clones stably expressing OVCA1 had a dramatic reduction in cyclin D1 levels (Fig. 7*B*). The reduction of steady-state cyclin D1 levels appeared to be primarily due to an increased rate of degradation of cyclin D1 in cells expressing HAOVCA1 compared with the parental cell line (Fig. 7*C*).

FACS Analysis of Stable Transfectants. Two main mechanisms, apoptosis and cell cycle arrest, may account for the growth suppression observed in stable clones expressing exogenous OVCA1. To investigate the mechanism of growth suppression, we seeded parental A2780 cells and each of the stable transfectants at an equal number of cells. Seventy-two h postseeding, the cells were harvested, nuclei were stained with ethidium bromide, and cell cycle distribution was measured by FACS analysis. As illustrated in Fig. 8, a 10–20% increase in the number of cells in the G_1 fraction was observed in clones OV-5, OV-9, and OV-13 compared with parental A2780 cells and stable vector control cells, CMV-5. No subdiploid cell peaks suggestive of apoptosis were observed. To further investigate the possibility of apoptosis playing a role in reduced cell number, we subjected clones OV-5 and OV-9 to TUNEL staining and compared them with the vector control cells. There were no TUNEL-positive cells on the vector control cell slides. A total of 1.2% of the OV-9 cells were TUNEL-positive, and 4% of the OV-5 cells were TUNEL-positive, suggesting that, although rates of apoptosis are slightly elevated in A2780 cells stably expressing OVCA1, apoptosis does not fully account for the drastic reduction in growth rates (data not shown).

Cyclin D1 Overexpression Can Partially Overcome OVCA1's Suppression of Clonal Outgrowth. Reduction of cyclin D1 levels by OVCA1 may be the primary cause of OVCA1's growth-suppressive

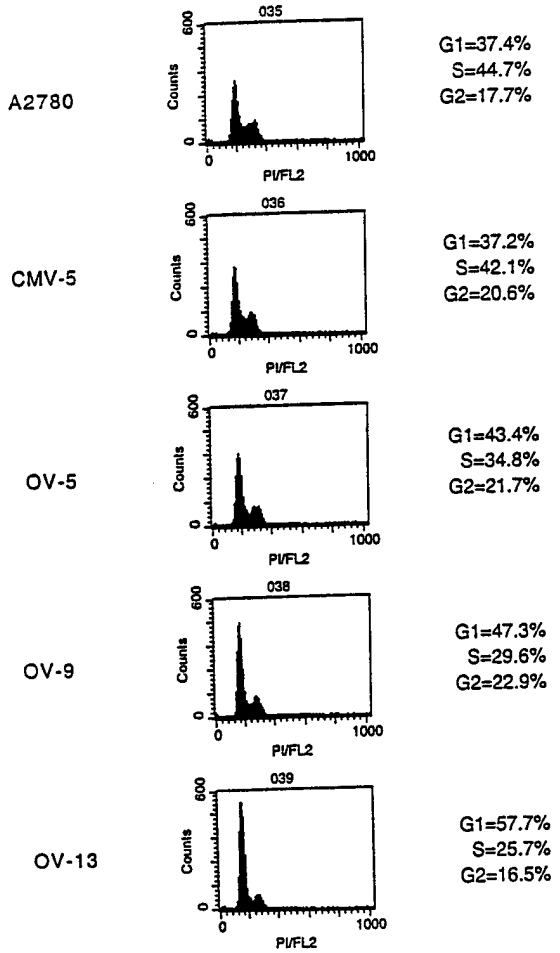

Fig. 8. FACS analysis of stable transfectants. Cell cycle distributions were determined 72 h postseeding by flow cytometry, as described in "Materials and Methods." DNA profiles represent cell number (counts) along the *ordinate* and DNA content (PI/FL2) along the *abscissa*. Percentage of cells in each phase of the cell cycle is listed on the *right*. G_1, both the G_0 and G_1 populations of cells; *S*, the population of cells in DNA synthesis; G_2, both the G_2 and M populations of cells.

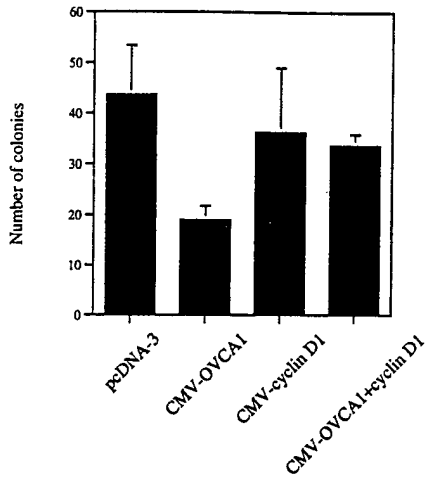

Fig. 9. Overexpression of cyclin D1 can override OVCA1's suppression of clonal outgrowth. A2780 cells were transfected with the indicated plasmids, as described in "Materials and Methods." Resistant colonies were selected in G418 for 14 days and then they were fixed and stained. Colonies with >50 cells were counted. The experiment was repeated three times. *pcDNA-3*, transfected with 1 μg of pcDNA3 and 1 μg of pskII; *CMV-OVCA1*, transfected with 1 μg of pcDNA3-OVCA1HA and 1 μg of pskII; *CMV-cyclin D1*, transfected with 1 μg of CMV-cyclin D1 (carries no selectable marker) and 1 μg of pcDNA3; *CMV-OVCA1+-cyclin D1* was transfected with 1 μg of pcDNA3-OVCA1HA and 1 μg of CMV-*cyclin D1*.

effect. To test this theory, we cotransfected A2780 cells with pcDNA3-*OVCA1HA* and CMV-*cyclin D1*. The cells were transfected with pcDNA3 alone, pcDNA3-*OVCA1HA* alone, CMV-*cyclin D1* alone, or both pcDNA3-*OVCA1HA* and CMV-*cyclin D1*. pcDNA3 and pskII were added as necessary to equalize the amount of selectable marker and plasmid DNA in each transfection. After selection for 14 days in G418, the colonies were fixed, stained, and counted. As noted previously, cells transfected with the OVCA1 expression construct formed ~50% fewer colonies than did cells transfected with pcDNA3 (Fig. 9). Cells transfected with the cyclin D1 expression vector formed almost as many colonies as did cells transfected with pcDNA3. Cells cotransfected with pcDNA3-*OVCA1HA* and CMV-*cyclin D1* formed ~75% fewer colonies than did cells transfected with pcDNA3 and almost the same number of colonies formed by cells transfected with CMV-*cyclin D1* alone, suggesting that overexpression of cyclin D1 can compensate for overexpression of OVCA1.

DISCUSSION

Molecular studies of human neoplasms suggest that a tumor suppressor locus exists on chromosome 17p13.3 near the VNTR markers YNH37.3 and YNZ22.2 (14–18, 20–25). To date, only two genes have been reported that map within the critical region of allelic loss on chromosome 17p13.3: *OVCA1* and *OVCA2* (47, 48). We have found that *OVCA2* cannot suppress tumor cell proliferation.[5] The amino acid sequence of OVCA1 contains little information with regard to its biological function. The only portion of the protein that is similar to previously identified proteins is a region in the NH_2 terminus that is similar to a domain found in a number of proteins isolated from a variety of species (47, 48). Unfortunately, the function of this domain is unclear. The only member of this putative gene family to which a function has been assigned is the yeast protein DPH2, which is known to play a role in the synthesis of diphthamide (56). It is unlikely that *OVCA1* is the human homologue of the yeast *dph2* because at least one other human gene, *DPH2L2*, is more similar to the yeast *dph2* than is *OVCA1* (57).

Screening of a panel of primary breast (n = 20) and ovarian (n = 50) tumors for alterations of *OVCA1* revealed two distinct missense changes and multiple polymorphisms in both the coding and noncoding regions. Both missense changes were detected in breast tumors, and each alteration was present in the germ line of a woman with a strong family history of this disease. In both cases, the missense mutation/rare polymorphism was retained in the corresponding breast tumor DNA and showed reduction to homozygosity. Evaluation of >100 control chromosomes failed to detect these sequence variants. The probands do not have unusual ancestries, indicating that the sequence alterations are unlikely to be related to a specific ethnic group. Unfortunately, the probands are deceased, and we do not have informed consent to contact other members of their respective families. Both of these probands have tested negative for germ-line mutations in BRCA1 and BRCA2.[6] However, neither amino acid substitution alters OVCA1's ability to suppress colony formation, suggesting that either these alterations are nonfunctional polymorphisms or that they affect some as yet undefined function of OVCA1 or perhaps alter the function of the p85 form of OVCA1. Our observation is of particular significance because, in a recent European Consortium study, an association between LOH at the *OVCA1* locus and a positive family history of breast cancer was observed (16).

We also assessed ovarian tumors for large alterations involving the *OVCA1* gene by Southern blotting; however, no rearrangements or large interstitial deletions were detected. One previous study has reported a homozygous deletion in an ovarian carcinoma that involved both D17S28 and D17S30 but not any other flanking markers (15). Overall, no somatic mutations were detected within the coding region of *OVCA1* at the DNA level in either primary breast or ovarian tumors.

Because *OVCA1* does not appear to be commonly mutated in tumors and tumor cell lines, we sought to determine whether changes in its protein levels are more frequent in breast and ovarian cancer. Western blot analysis of extracts from breast and ovarian tumors suggest that expression of p50 OVCA1 is reduced in at least one-third of the tumor specimens evaluated. The larger putative forms of OVCA1 (p70/p85) are absent or highly reduced in almost 100% of the tumor specimens evaluated. The mechanism whereby the p70/p85 forms of OVCA1 are generated is as yet unclear. Several different antibodies raised against different regions of OVCA1 recognize the larger isoforms, confirming that they are closely related to the p50 OVCA1. Most likely, the p85 form is the product of an as yet undefined alternatively spliced exon or posttranslational modification. The p70 form is not recognized by antibodies directed against the COOH terminus of OVCA1, suggesting that it is either a degradation product of the p85 form or an unrelated, cross-reacting protein. If reduction of OVCA1 levels is important in tumorigenesis, then reintroduction of *OVCA1* into tumor cell lines should revert, at least partially, the transformed phenotype. Because the p85/p70 isoforms are most consistently lost from tumors, reintroduction of these forms would be most informative. However, because they have not yet been completely defined, our experiments were confined to reintroduction of the p50 isoform. Attempts to stably express OVCA1 from the CMV promoter in a variety of cell lines were unsuccessful. This phenomenon crossed species lines, being apparent in cells derived from both rodents and primates; was independent of p53 status; and was evident in both immortalized and transformed cells, suggesting that overexpression of OVCA1 either blocks growth or is toxic to the cells.

Overexpression of OVCA1 in the ovarian cancer cell line A2780 provided some clues about the function of OVCA1. It was possible to

[5] T. White and A. Prowse, unpublished observations.

[6] A. K. Godwin, unpublished observations.

isolate a few clones that expressed exogenous OVCA1. In all cases, the level of exogenous expression was not high and was, at most, equivalent to the amount of OVCA1 normally seen in A2780 cells. Cells expressing exogenous OVCA1 were found to have a 4-fold (OV-9) to 10-fold (OV-13) reduction in growth compared with parental A2780 cells. The cellular mechanisms by which OVCA1 suppresses growth could fall into three categories: apoptosis, replicative senescence, or cell cycle arrest. On the basis of the amino acid sequence of OVCA1, it is unclear which, if any, of these pathways OVCA1 may affect. It is unlikely that OVCA1 promotes cell death to any great extent. TUNEL labeling and FACS analysis suggest that, although the OVCA1 stably expressing clones have a slightly elevated rate of apoptosis, it is not significant enough to account for the dramatic reduction in proliferation rates. It is also unlikely that exogenous OVCA1 restores replicative senescence because stable overexpression of OVCA1 did not affect rates of colony outgrowth (data not shown).

Cell cycle analysis of the OVCA1 stably expressing clones suggests that decelerated growth was associated with an increased percentage of the population in the G_0-G_1 phase of the cell cycle. We observed a reduction of cyclin D1 levels caused by destabilizing the protein, and this may be the direct cause of the slowed proliferation rates. In support of this hypothesis, cotransfection of cyclin D1 was able to override OVCA1's suppression of clonal outgrowth. Cyclin D levels are primarily regulated at the transcriptional level in response to extracellular mitogenic stimulation; however, in the absence of such stimulation, cyclin D is rapidly degraded by calpain proteases (Ref. 58; reviewed in Ref. 59). It is not yet clear as to how increased levels of OVCA1 leads to destabilization of cyclin D1. Deregulation of cyclin D1 has been implicated in the generation of many types of tumors. In some tumors, overexpression of cyclin D1 is achieved by amplification of the *cyclin D1* gene (reviewed in Ref. 60). However, in other tumors, including ovarian tumors, overexpression of cyclin D1 is not associated with genetic alterations, suggesting that some other mechanism, perhaps an increase in stability, is the cause of the abnormality (61, 62).

Analyses of ovarian and other tumors clearly indicate that allelic loss of chromosome 17p13.13 is one of the more frequently observed molecular alterations; >70% of ovarian tumors, at least two-thirds of breast tumors, and many other types of tumors have lost part or all of one copy of chromosome 17 (see "Introduction"). It was previously thought that both alleles of a tumor suppressor gene must be inactivated, as addressed by the "two-hit" hypothesis for tumorigenesis of Knudson (63). However, it has recently been shown that genes such as the murine gene *p27/kip1* and the *PTEN* gene are haploinsufficient for tumor suppression (64, 65). Abnormally low levels of the p27 protein are frequently found in human carcinomas (66–70). However, it had never been possible to establish a causal link between p27 and tumor suppression because only rare instances of homozygous inactivating mutations of the *p27* gene were found in human tumors (71–74). However, it was shown that both *p27* nullizygous and *p27* heterozygous mice were predisposed to tumors in multiple tissues when challenged with γ-irradiation or a chemical carcinogen (64). Molecular analyses of tumors in *p27* heterozygous mice showed that the remaining wild-type allele was neither mutated nor silenced (64). The *PTEN* gene encodes a dual-specificity phosphatase mutated in a variety of human cancers (75–77). *PTEN* germ-line mutations are found in three related human autosomal dominant disorders, CD, LDD, and BZS, characterized by tumor susceptibility and developmental defects (78–80). It was recently reported that *PTEN*+/− mice and chimeric mice derived from *PTEN*+/− embryonic stem cells showed hyperplastic/dysplastic changes in the prostate, skin, and colon, which are characteristic of CD, LDD, and BZS, respectively (65). They also spontaneously developed germ cell, gonadostromal, thyroid, and colon tumors, suggesting that *PTEN* haploininsufficiency plays a causal role in CD, LDD, and BZS (65). These studies, therefore, suggest that there is another class of tumor suppressor genes, in which genes that exhibit haploinsufficiency, leading to reduced levels of the protein, are important for tumorigenesis.

The data presented here and elsewhere, *i.e.*, high rate of allelic loss observed for chromosome 17p13.3 in ovarian tumors, the reduced expression of OVCA1 in ovarian tumors, and the observation that an equimolar level of exogenous p50 OVCA1 suppresses the growth rate of tumor cells up to 10-fold, suggest that a slight reduction in the level of expression of OVCA1 is sufficient for loss of growth regulation. The high rate of loss of one copy of chromosome 17p in breast and ovarian tumors may contribute to carcinogenesis by reducing *OVCA1* to hemizygosity. Future efforts aimed at clarifying the biochemical function of OVCA1 will aid in confirming the role that this gene has in tumorigenesis as well as its normal cellular function.

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APPENDICES – PROJECT II

APPENDIX A

MANUSCRIPT

Gynecologic Oncology, in press

Decision Making about Prophylactic Oophorectomy among At-Risk Women: Psychological Influences and Implications

Suzanne M. Miller, Ph.D., Carolyn Y. Fang, Ph.D., Sharon L. Manne, Ph.D.,

Paul F. Engstrom, M.D., and Mary B. Daly, M.D., Ph.D.

Fox Chase Cancer Center

Philadelphia, Pennsylvania

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Address correspondence concerning this article to Suzanne M. Miller, Ph.D., Division of Population Science, Fox Chase Cancer Center, 510 Township Line Road, Third Floor, Cheltenham, PA 19012. Tel: 215-728-4069. Fax: 215-728-3056. Electronic mail may be sent to SM_Miller@fccc.edu.

Running Head: **DECISION MAKING ABOUT PROPHYLACTIC OOPHORECTOMY**

Abstract

Objective: Women with a family history of ovarian cancer are confronted with difficult decisions regarding the management of their risk status. Currently, the main preventive option available is prophylactic oophorectomy. The objective of the present paper is to review research and theory on psychological factors that influence decision making about preventive surgery and discuss the implications for patient management. Methods: Guided by a cognitive-social framework, the literature on decision making about preventive surgery is reviewed and integrated. Results: The available studies show that women are more likely to opt for surgery if they feel more vulnerable to cancer, believe that surgery will prevent cancer, and are worried about developing cancer. Further, the response to ovarian risk is influenced by the individual's characteristic psychological style: monitors (who typically scan for and amplify threatening cues) tend to feel more vulnerable to cancer and more distressed about their cancer risk than blunters (who typically distract from threatening cues) do. Conclusion: On the basis of prior research, monitors may be more likely to choose surgical intervention to reduce their distress, without fully anticipating the psychological and medical consequences of that decision. In order to facilitate informed decision-making, counseling protocols should be designed to enable the patient to understand, and take account of, the psychological consequences of the available medical options. Future studies are needed to systematically extend and explore the proposed theory-based relationships.

Key words: ovarian cancer risk, prophylactic oophorectomy, monitoring vs. blunting

Overview

Ovarian cancer is associated with the highest mortality rate among all of the gynecological cancers [1], resulting in more than 14,500 deaths each year in the United States [2]. The high incidence of ovarian cancer-related mortality is believed to be due to two main factors. First, no distinctive symptoms have been identified in patients at the early stages of disease [2,3]. Second, currently available surveillance methods have not proven to be highly reliable in detecting early-stage disease [2,3]. The challenges posed by the limitations of early detection are of particular concern in the case of patients at increased familial risk for ovarian cancer [4]. One medical strategy being offered to these women is prophylactic oophorectomy, that is, the surgical removal of healthy ovaries [4,5]. The information that needs to be conveyed about this preventive option is complex, making it difficult for patients to accurately weigh the costs and benefits of alternative choices.

To date, few empirical data are available on how at-risk women understand, and make decisions about, prophylactic surgery. As a result, little is known about how to communicate necessary information in a manner that optimizes decision making and enhances patient adaptation to the decisions made. In the present paper, we briefly review the medical issues facing women at familial risk for ovarian cancer, particularly with respect to their preventive options. We describe a cognitive-social theoretical framework that delineates the psychological factors that play a role in the decision-making process. We then illustrate two prototypic styles of processing ovarian cancer risk feedback, monitoring vs. blunting, and describe how they influence and interact with the psychological factors that influence decision-making processes. Finally, we discuss the

implications of the current findings for counseling protocols designed to enhance decision making about prophylactic oophorectomy.

Preventive Options for Ovarian Cancer Risk

Epidemiological evidence has identified family history as one of the major risk factors for ovarian cancer [2,6]. First-degree relatives (FDRs: i.e., mother, sister, or daughter) with one affected family member have a lifetime risk of 5%, which is more than three times the 1.4% lifetime risk for women without a family history [2,7]. For women with two affected family members, the lifetime risk rises to 7% [2]. Further, women who have a genetic susceptibility to breast and/or ovarian cancer (i.e., who are carriers of a *BRCA1/2* genetic mutation) have a 16% to 65% lifetime risk of developing ovarian cancer [8].

Patients presenting with localized disease have a 79% five-year survival rate. Yet, despite significant interest in improving early detection of ovarian cancer [6], 75% of all ovarian cases present with advanced stage disease [9]. Advanced stage disease is difficult to treat effectively and is associated with an alarmingly low 5-year survival rate, of approximately 28% [10]. Contributing to this high mortality rate are two factors: 1) the absence of well-recognized signs and symptoms during the early stages of disease; and 2) the fact that the available surveillance methods have relatively poor sensitivity and specificity [2].

Since effective detection and management strategies for ovarian cancer are limited, preventive options become important, particularly for women at increased risk for disease. Current methods include the use of oral contraceptives and tubal ligation [2,6,11-14]. For example, oral contraceptive use for six or more years is associated with a 60 percent reduction in risk among

women who carry *BRCA1* or *BRCA2* mutations [15]. However, the studies conducted to date have not resolved the issue of whether the potential benefits (i.e., ovarian cancer risk reduction) outweigh the possible risks (i.e., increased breast cancer risk; [16-19]) for high-risk women. Hence, these approaches have not been routinely incorporated into standard care.

A primary surgical preventive option available for high-risk women is prophylactic oophorectomy, that is, the surgical removal of noncancerous ovaries [2,4,20,21]. Studies have shown that prophylactic oophorectomy significantly reduces ovarian cancer risk in pre-, as well as post-, menopausal women [22,23]. It has been estimated that a 30-year old woman with hereditary breast-ovarian cancer syndrome can gain from 0.3 to 2.6 additional years of life expectancy as a result of prophylactic oophorectomy [24,25]. A recent study using a Markov model showed that high-risk women (i.e., those with an affected relative and a positive *BRCA1/2* mutation status) would live longer if they undergo prophylactic surgery [25]. On the other hand, the benefits of prophylactic surgery appear to be small or nonexistent for women at lower risk [25]. Gains in life expectancy decline with age at the time of surgery, and appear to be minimal for women 60 years of age and older [24].

Along with the potential medical benefits, patients inclined to undergo prophylactic oophorectomy must also consider the potential limitations of the procedure [2,6,20,26]. First, the surgery does not appear to completely eliminate cancer risk. Although the data are limited, cases of post-oophorectomy intra-abdominal carcinomatosis (which histologically resembles ovarian cancer) have been reported in the literature [23,26,27]. Like ovarian cancer, peritoneal cancers are also difficult to detect at an early stage, and thus, women contemplating prophylactic

oophorectomy need to consider whether they will continue to feel vulnerable to cancer, even after they have had their ovaries removed [28].

Second, the surgical procedure itself is associated with certain risks (e.g., surgical morbidity and post-surgical complications), particularly for those women who are not candidates for laparoscopic surgery. Further, surgery can entail a lengthy hospital stay and recuperative period, and may be complicated by adhesions and small bowel obstruction. Third, estrogen deprivation following prophylactic oophorectomy results in an elevated risk for heart disease and osteoporosis [29]. To counteract these effects, patients are advised to undergo a prolonged regimen of Hormone Replacement Therapy (HRT). HRT may be associated with increased risk for breast cancer [30-32], which may raise anxiety and interfere with compliance. Indeed, published reports suggest that between 11% and 69% of women are noncompliant with HRT [33,34]. Fourth, for women of reproductive age, the loss of future childbearing potential may represent a source of emotional distress [28].

Women who are inclined to forego prophylactic oophorectomy need to consider two main potential limitations. First, they may have to deal with sustained perceptions of vulnerability, since available detection methods are not highly reliable [2,6]. Second, the necessity of undergoing repeated ovarian screening (e.g., a bimanual rectovaginal examination, transvaginal ultrasonography with Doppler flow, and serum blood testing for the antigenic CA-125 tumor marker) may cause distress, as the surveillance may serve as a continuous reminder of one's vulnerability to disease. Given that there is no medically "right" or "wrong" preventive recommendation for at-risk women, the decision about whether or not to undergo prophylactic

oophorectomy needs to take in-depth account of the psychological consequences of each option for a given individual [35].

A Cognitive-Social Theoretical Framework for Decision Making About Prophylactic Oophorectomy

Decades of research have shown that individuals make judgments about how to manage perceived health risks in ways that cannot be understood primarily in terms of the statistical considerations on which rational decision-making models are based [36,37]. This is particularly likely to be the case when the information they receive is emotionally threatening and the stakes are highly personal and entail significant threats to one's sense of well-being [38-40]. In the context of genetic testing for breast and ovarian cancer risk, for example, the results show that women often focus selectively on the potential benefits (e.g., gaining reassurance) and ignore the potential limitations (e.g., continued anxiety, regret) of genetic risk feedback [40-44].

The Cognitive-Social Health Information Processing (C-SHIP) model [45-48] provides a theory-based framework for guiding the application of behavioral science to understanding how at-risk women deal with the decision-making process [49-53]. The cornerstone of this approach is that a woman's decisions are determined by how she cognitively and emotionally processes information about her cancer risk [e.g., 49,54-57]. In this approach, decision making is influenced by three main factors: 1) how the patient construes her vulnerability to disease (i.e., her perceived susceptibility to ovarian cancer); 2) the patient's expectancies and beliefs about the efficacy of available courses of action (i.e., the advantages and disadvantages of prophylactic oophorectomy and repeated surveillance); and 3) the patient's affects and feelings (i.e., her worries and

concerns). We now review evidence for the role of these factors in decision making about prophylactic oophorectomy, citing literature where relevant studies exist, and drawing from related literature in instances where direct evidence is not yet available.

Health-Relevant Encodings

Health-relevant encodings refer to how an individual appraises incoming threat and disease-relevant information (e.g., cancer risk feedback) [48]. These encodings play a role in decision making about preventive surgery among high-risk women. For example, a significantly higher percentage of women who test positive for a *BRCA1* genetic mutation (which has been found to increase perceptions of vulnerability) express interest in prophylactic oophorectomy than those who test negative (76% vs. 0%; [58]). In a descriptive study of relatives from *BRCA1* families, decision making about undergoing prophylactic oophorectomy was cited as one reason for undergoing genetic testing [58]. Indeed, in a study of FDRs of breast cancer patients, women who tested positive for a *BRCA1* mutation were more inclined to consider prophylactic oophorectomy than prophylactic mastectomy [59]. The focus on prophylactic oophorectomy is understandable, given the current limitations of early detection and surveillance regimens for ovarian disease [2]. In related work with FDRs of breast cancer patients, women who expressed an interest in prophylactic mastectomy perceived their risk of disease to be higher than women who were not interested in surgery [60].

Health-Relevant Expectancies, Beliefs, and Values

Health-relevant expectancies refer to the individual's self-efficacy beliefs (e.g., "I am able to comply with ovarian cancer screening recommendations"), as well as to the anticipated

consequences of particular courses of action (e.g., "Undergoing prophylactic oophorectomy will reduce my chances of getting ovarian cancer"). Individuals' health values refer to the personal importance that is placed on various health outcomes, such as the ability to have children. These expectancies, beliefs, and values can have profound consequences for health behaviors [61,62].

Health behaviors are influenced by the outcome and efficacy expectancies with regard to available courses of action, as well as the perceived quality of early detection, prevention, and treatment consequences [48]. In one study, women were highly likely to consider prophylactic oophorectomy if they believed that it would reduce their ovarian cancer risk and provide the only means by which they could guarantee their survival, and thereby enable them to fulfill their social obligations [28]. On the other hand, women were less inclined to consider prophylactic oophorectomy if they believed it would upset the natural balance of their body, if they questioned the efficacy of the procedure, if they believed the operation would compromise their social obligations, or that it would result in immediate cessation of fertility [28].

Affect

Women's cancer-related worries and anxieties contribute to their decisions regarding cancer prevention options. Studies of women at risk for ovarian cancer have found that they experience moderate to high levels of psychological distress [63,64], low perceptions of control, and elevated cancer risk perceptions. For example, among 154 women with a familial history undergoing surveillance for ovarian cancer, a significant proportion (31.4%) reported experiencing high levels of depressive symptoms and 16% exhibited elevated levels of anxiety [64].

The affective consequences of the individual's cancer risk status appear to have implications

for her decisions regarding prophylactic surgery. Notably, women who are more worried about their breast cancer risk are also more interested in prophylactic mastectomy than are women who are less concerned [65]. Case reports also cite higher levels of anxiety in at-risk women who choose to undergo prophylactic mastectomy versus those who decline preventive surgery [66]. Thus, affective factors (e.g., worry) appear to influence women's decision making processes in favor of preventive surgery.

Information Processing Styles: Monitoring versus Blunting of Ovarian Cancer Risk

The literature reviewed above suggests that psychological factors influence women's decision making about prophylactic oophorectomy. In particular, the available findings indicate that heightened perceptions of vulnerability to ovarian cancer, as well as greater worries about ovarian cancer, are associated with greater interest in preventive surgery. Further, preliminary data indicate that women hold positive or negative expectancies regarding the outcome of the surgery, and these expectancies may be a factor in women's decision making.

Previous research has identified two main cognitive-affective processing styles that people use to deal with medical threats: monitoring versus blunting. The first processing style, monitoring, is characterized by scanning for, and amplifying, threatening cues. The second processing style, blunting, involves distraction from threatening cues [67]. Individuals with these types of information processing dynamics have been identified with the Monitoring-Blunting Style Scale (MBSS), for which extensive evidence is available [67]. In contrast to blunters, monitors tend to respond to cancer threats with higher levels of perceived vulnerability, lower levels of perceived self-efficacy and control, and heightened cancer-related distress [61,68,69].

In the ovarian risk context, monitors have been found to have increased perceptions of vulnerability to the disease, since they tend to scan for, and attend to, health threats pervasively [67]. In a study of first degree relatives (FDRs) of ovarian cancer patients, monitors perceived themselves to be at greater risk for developing the disease than blunters, independent of their true levels of risk [69]. Increased perceptions of risk and accompanying intrusive ideation, in turn, can undermine adaptive health-protective behaviors by leading to increased levels of distress [70,71].

Monitors and blunters also differ in their expectations of how genetic risk information will impact on them. Lerman and colleagues [68] examined interest in, and expectations about, the impact of genetic testing among 121 women who had a first-degree relative (FDR) with ovarian cancer. Overall, the majority of women (75%) reported being "definitely interested" in genetic testing. However, monitors anticipated that they would react more negatively to testing feedback than blunters. That is, monitors believed that genetic testing feedback would make them more depressed and anxious in comparison with blunters [68]. Thus, although monitors expressed greater interest in knowing or learning more about their cancer risk, they also anticipated that they would respond more adversely to the psychological consequences of this information [68].

Finally, monitors and blunters have been found to differ in their affective response to ovarian cancer risk. Wardle and colleagues [72] studied at-risk women in a screening program to detect early familial ovarian cancer by ovarian ultrasound. Distress was measured before and after their first screening using the General Health Questionnaire (GHQ). Women were informed of any abnormality immediately; none of the patients were ultimately found to have ovarian cancer. Before the scan, all groups showed equivalent levels of distress. After the scan, monitors with

positive (i.e., abnormal results) showed greater increases in distress compared with blunters receiving positive results and compared with patients receiving negative (i.e., normal) results. Among those undergoing follow-up scans for positive results, monitors who again tested positive showed a greater increase in anxiety than other women. Moreover, these effects were long-lived [73]. One year after having had a false positive result, monitors reported significantly higher levels of distress and anxiety (as measured by the GHQ) than blunters. Further, monitors who underwent surgical intervention showed the highest levels of distress as measured by the GHQ [73].

Conclusions and Future Directions

For the foreseeable future, a key preventive strategy for women at familial risk for ovarian cancer will continue to be prophylactic oophorectomy. Consistent with the cognitive-social framework, the available literature shows that health-relevant encodings, expectancies, and affect are related to women's decision-making processes. Specifically, patients are more likely to opt for prophylactic oophorectomy when they feel highly vulnerable to cancer [58,60], perceive that surgery will be effective in preventing cancer [28], and are highly distressed about their cancer risk [65,66]. This pattern of reactions may undermine informed decision-making, by prompting individuals to impulsively opt for preventive surgery without fully considering the benefits and limitations of the procedure. However, it should be noted that the associations observed in prior studies have yet to be prospectively examined. Further, the research conducted to date has focused primarily on women's intentions to undergo prophylactic surgery, rather than on women's actual decision making processes and subsequent behavioral choices. There is also a need for

longitudinal studies to explore the correlates and consequences of these relationships over time.

The data reviewed may have implications for the development of counseling protocols. Specifically, patients may need to be helped to take account of the psychological consequences of alternative options for them personally [74]. That is, informed decision making may require that potential candidates be able to realistically process and anticipate the benefits, as well as the limitations, of undergoing preventive surgery [48,74]. At present, existing guidelines do not deal with how to convey information to patients so as to facilitate decision making and to enhance subsequent adaptation to the scenarios that unfold [74]. Further, the psychological factors that undermine the effective utilization of risk information have not received systematic attention, particularly in the case of healthy women contemplating prophylactic oophorectomy.

Traditionally, counseling programs have focused on improving the comprehension of cancer risk feedback and educating patients about their options [59,75]. One approach has been to offer personalized cancer risk counseling to women, based on their specific familial, reproductive, and other personal risk factors [e.g., 75,76]. The results show that women who receive personalized risk feedback are significantly more likely to accurately estimate their risk and to report reductions in cancer-specific distress, compared to women who receive general health feedback [59,75]. Yet, two-thirds of women continue to overestimate their risk for cancer [75]. Hence, merely providing education and information about a medical procedure or test is not sufficient for optimal decision making [77]. Thus, counseling interventions may be needed that explicitly address the cognitive and affective barriers that undermine informed decision making.

The findings also suggest that, in addition to providing personalized risk feedback, counseling

interventions may need to be tailored to the individual's psychological profile. Monitors tend to overestimate their vulnerability to cancer [69] and to experience increased levels of disease-related distress and anxiety [69,72]. Blunters, in contrast, tend to feel less vulnerable to cancer and to manifest lower levels of distress [48,78]. Specifically, monitors tend to perceive themselves to be more vulnerable to cancer, have more negative expectancies about one's cancer risk status, and experience more distress about their cancer risk compared with their blunting counterparts. Further, findings in other cancer models show that outcomes are improved when the individual's attentional style is explicitly targeted in intervention communications [69,79-81]. Future work should more systematically extend research on monitoring-blunting attentional style to decision making about ovarian cancer risk. In particular, there is a need for studies that explore whether monitors benefit from interventions that inform them about the potential limitations of prophylactic oophorectomy and provide support for the complex emotional reactions that may be triggered, and whether blunters benefit from interventions that orient them to the possible advantages of the procedure [69,80,82].

The principles that need to be tested and the techniques that need to be developed for informed decision making are relevant not only to the medical and psychological management of ovarian cancer risk, but may also lay the groundwork for cancer prevention counseling protocols for other groups of at-risk individuals [83]. Ultimately, the findings of this type of research should fill a theoretical and empirical gap by providing a framework for specifying how to systematically prepare at-risk individuals for decision making, tailored to the distinctive psychological profile of the patient. This, in turn, should improve a range of patient outcomes,

including decision-making, satisfaction, quality of life, and adherence over the long-term.

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Article Precis

Using a cognitive-social framework, the article reviews theory and empirical evidence on psychological factors that influence decision making about prophylactic oophorectomy.

APPENDIX B

MANUSCRIPT

Anxiety/Uncertainty Reduction as a Motivation for Interest in Prophylactic Oophorectomy in Women with a Family History of Ovarian Cancer

Karen E. Hurley, Ph.D., Suzanne M. Miller, Ph.D, Josephine W. Costalas, MS, Mary B. Daly, MD, Ph.D. (Fox Chase Cancer Center)

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Correspondence: Karen Hurley, Ph.D., Ruttenberg Cancer Center, Mt. Sinai School of Medicine, 1425 Madison Ave., #1130, New York, NY 10029, 212-659-5520

Running Head: ANXIETY REDUCTION AND INTEREST IN OOPHORECTOMY

Abstract

Purpose: Most women with a family history of ovarian cancer must decide about prophylactic oophorectomy (PO) without conclusive information about their risk level and without clear-cut efficacy data about PO. Some women with relatively low-risk profiles seek PO or are recommended the procedure by their physicians if they appear "cancerphobic." This study investigated the relation of cancer anxiety and other factors to interest in PO in a group of women with varying degrees of familial risk for ovarian cancer.

Patients and Methods: Patients were 94 women enrolled in an ongoing program for women with a family history of ovarian cancer. Patients received personalized risk counseling and were classified as having a sporadic, familial, or hereditary pedigree by a genetics counselor. Eligible enrollees were interviewed by telephone about current and future interest in PO, perceived risk of ovarian cancer, cancer anxiety, stress-related ideation, and reasons for and against surgery.

Results: Half of the women reported current interest in PO. Reduction of anxiety/uncertainty was the factor most strongly associated with current interest in PO, independent of objective risk classification, perceived risk, cancer anxiety or stress-related ideation. Future interest in PO was predicted by other perceived benefits of surgery.

Conclusions: Current, but not future, interest in PO appears motivated in part by seeking immediate relief from anxiety. Interest may fluctuate based on varying exposure to cues that trigger anxiety and on development of alternate coping strategies. Women seeking PO, particularly those with low-risk profiles, should be offered options for anxiety management as part of informed consent for PO.

INTRODUCTION

Ovarian cancer is one of the most lethal gynecological cancers. The overall survival rate is only 50%, compared with breast (86%), cervical (72%) and endometrial (87%) cancers .[1] Researchers estimate that, in 1999, approximately 25,200 new cases of ovarian cancer will be detected in the United States, and 14,500 women will die from the disease [1]. The strongest risk factor for ovarian cancer is a family history of the disease [2], which confers a 5-7% lifetime risk in women with one or two affected first-degree relatives (compared to a 1.5% risk for women with no family history) and a 6-60% risk for those who with an inherited genetic mutation [3;4]. Few choices for risk management exist for women with a family history of ovarian cancer. The screening tests are that are available (transvaginal ultrasonography and blood testing for the tumor marker CA-125) fall short in sensitivity and specificity compared to screening tests used to detect other gynecological cancers [5;6]. Other than chemoprevention, which has not moved beyond the investigation stage due to questions about efficacy and side effects, the main option presently available to reduce ovarian cancer risk is bilateral prophylactic oophorectomy (PO), or surgical removal of non-cancerous organs in order to prevent occurrence of the disease.

Little is known about how women make the decision whether to undergo PO. The NIH consensus panel on ovarian cancer has recommended PO for women who appear to have a hereditary ovarian cancer syndrome (as determined through genetic testing, if available, or by the following family history criteria (vertical transmission, occurrence at a young age, multiple cases

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[1]These figures are for Caucasian women living in the U.S. Comparable figures for African American women average 16.5 percentage points lower [1].

of ovarian cancer in the family, or breast and ovarian cancer in the same family) and who have either completed childbearing or reached the age of 35[7]. Most women with a family history of ovarian cancer do not meet these criteria and must make decisions about PO without having conclusive information about their level of risk.

The sparse literature to date on prophylactic surgery indicates that anxiety and worry play a key role in the decision-making process. A study of 164 women presenting at a breast clinic showed that worry about developing cancer was the sole predictor of whether women underwent prophylactic mastectomy. Although generalizations to oophorectomy must be made with caution, one recent report[8] provides anecdotal evidence that women undergoing genetic counseling perceived one of the benefits of prophylactic oophorectomy to be as a means to manage their anxiety about cancer. Anxiety is also viewed by some medical practitioners as an indication for surgery, who will recommend the procedure if their patients appear "cancer-phobic" even if they do not have a marked family history.[9;10]

Treating a woman's anxiety about cancer through prophylactic surgery incurs medical consequences, both through the immediate impact of surgery and its risks, and through the long-term effects of surgical menopause. Although no prospective studies of the efficacy of PO have been carried out, the literature to date suggests that the procedure can reduce, but does not completely eliminate risk of ovarian cancer[11-13]. Furthermore, PO raises the issue of starting hormone replacement therapy (HRT) to address the increased risk of heart disease or osteoporosis associated with prolonged estrogen depletion[14]. Taking HRT after a prophylactic oophorectomy can raise one's risk of breast cancer, especially if the hormone is taken for more

than ten years[15], and also increases the density of breast tissue, making mammography a less sensitive test[16], crucial points for women with family histories of both breast and ovarian cancer.

In order to help women come to a decision that balances concerns about their long-term physical and psychological well-being, it is important to determine the relative salience of anxiety compared to other reasons for and against surgery. Other factors that have been reported to influence women's decisions about PO include: reducing one's risk, maximizing one's chances of survival in order to meet family obligations, not wanting to upset the body's natural balance, fatalism that cancer will occur anyway, fears about the risks of surgery, worries about menopause and HRT, concerns about being able to meet family and work responsibilities while recuperating, childbearing, and identity issues.

Individuals will differ in the level of anxiety engendered by their risk status and how heavily they weigh anxiety compared to other factors[17]. Anecdotally, some women with a family history of cancer become convinced that they have a 100% chance of developing the disease and think of themselves as "walking time-bombs"[18]. The research on adjustment to ovarian and breast cancer risk suggests that approximately 15-25% of women with a family history of cancer are highly anxious about their chances of developing the disease and experience repeated intrusive thoughts about their risk, to the extent that it may interfere with daily activities[19;20]. This represents a sizeable group of women who may be interested in surgery primarily for management of their anxiety.

For women whose family history suggests an inherited susceptibility to cancer, anxiety can have a negative impact on ability to give informed consent to PO. These women may not fully

process information about the consequences of PO, including HRT, the need for ongoing screening, or continued cancer risk, both in the peritoneum and in other organs. It is also important to examine whether inflated subjective risk estimates and elevated distress contribute to consideration of prophylactic surgery among women with less marked family histories. Many women in this situation do overestimate their risk of cancer[20], and these inflated estimates are resistant to modification even after individualized risk education[21].

We conducted our study to determine interest in prophylactic surgery and its relation to anxiety (both cancer-specific anxiety and intrusive thoughts about one's level of risk) in a sample of women with varying degrees of familial risk for ovarian cancer who have not undergone genetic testing. The specific aims of the present study were to: 1) describe levels of current and future interest in prophylactic oophorectomy in women at varying degrees of familial risk for ovarian cancer; 2) characterize levels of cancer anxiety and stress-related ideation in women at varying degrees of familial risk for ovarian cancer; 3) determine the relative weight of anxiety reduction compared to other reasons for and against surgery; and 4) explore the relations of objective risk, perceived risk, anxiety, and anxiety-reduction as a motivation for prophylactic surgery. Our predictions were that there would be a wide range in levels of interest in prophylactic surgery; that there would a subgroup of women who were highly distressed about their risk; that anxiety reduction would rank highly as a reason in favor of seeking prophylactic surgery; and that high levels of anxiety, stress-related ideation, and desire to reduce cancer-related anxiety would be associated with interest in prophylactic surgery, regardless of familial risk classification.

METHODS

Overview and Background

The present study is part of a larger, ongoing clinical research effort being conducted with women who have a family history of breast and/or ovarian cancer through the Family Risk Assessment Program (FRAP) at Fox Chase Cancer Center. FRAP participants with a family history of at least one relative with ovarian cancer were invited to complete an interview on prophylactic ovarian surgery as a supplement to their regular, ongoing participation in FRAP. The present study combined a subset of baseline data previously collected from participants on entry into FRAP with data collected during the prophylactic oophorectomy (PO) telephone interview developed specifically for this study.

FRAP was founded in 1991 for women over the age of 25 with at least one first-degree relative (mother, sister, daughter) with breast or ovarian cancer. Initially, women were recruited by contacting relatives of patients being treated for breast or ovarian cancer at Fox Chase Cancer Center. Participants are now also self-referred, or are referred by their physician. After enrolling, FRAP participants attend a two-hour small-group education session on breast and/or ovarian cancer risk, and on the roles of cancer screening and preventive surgery in risk management. Nutritional assessment and dietary recommendations are also made. Each woman meets individually with a genetics counselor who reviews the woman's family history and provides a personalized risk estimate. Women are also instructed in breast self-examination and are offered screening tests, including mammography, transvaginal ultrasound, and CA-125 testing at the Fox

Chase Cancer Center facilities.

Procedure

FRAP participants with at least one first-degree relative with ovarian cancer and who had been enrolled in FRAP for at least one year were notified by letter that a study was being conducted to obtain additional detail on levels of interest in prophylactic surgery among FRAP participants. The letter stated that they would receive a telephone call inviting them to participate in a short, fifteen-minute interview. Informed consent was obtained in writing and over the phone. Women who agree to participate in the proposed study were interviewed by phone by the first author or another graduate level clinical interviewer using the measures listed below.

Baseline Measures

The following information was drawn from the FRAP database. These measures were collected upon the participant's entry into FRAP at least one year before the present study.

Demographics: age, ethnicity, marital status, and education level.

Objective risk of cancer. Participants' family history was categorized as hereditary, familial or sporadic by a medical genetics counselor from Fox Chase Cancer Center (hereditary=pattern of cancers in two or more generations, in 3 or more family members, fitting a vertical pattern of inheritance; familial=pattern of cancers in one or more generations, but not fitting a vertical pattern of inheritance; sporadic=single occurrence of a cancer). In cases where a participant had a history of cancer on both paternal and maternal sides of her family, she was assigned to the higher of the two risk categories.

Interview Measures

The following measures were collected in the telephone interview:

Interest in prophylactic ovarian surgery: Levels of interest were assessed with two questions: "At this time how strongly are you considering prophylactic surgery?" (current interest), and "How likely is it that you will have surgery someday?" (future interest), each of which was measured on a five-point scale (1=not at all, 5=very strongly/definitely). Participants who rated their interest as less than five were asked if they had considered surgery more strongly in the past, and indicated their past interest on a similar five point scale.

Perceived risk of developing ovarian cancer: Participants estimated their personal level of risk on a scale of 0-100.

Ovarian cancer anxiety. Participants were asked to rate how anxious they became when they thought about ovarian cancer on a single five-point item (1=not at all, 5=extremely).

Stress-related ideation: We used the intrusive ideation subscale of the revised Impact of Events Scale (RIES)[22]. This seven-item measure assesses intrusive thoughts, images, dreams and distress at reminders of a specified stressor. For the present study, participants were asked to respond to each item with respect to their familial risk for ovarian cancer. This instrument has been used by other researchers to assess threat-related ideation in patients at risk for cancer[19;23;24]. Participants rate the frequency of symptoms on a weighted 4-point scale (0=not at all, 1=rarely, 3=sometimes, 5=often). Scores can range from 0 to 35. Internal reliability of the scale when has been assessed in non-medical (.78) and cancer populations (.84; Lerman et al., 1996). Test-retest reliability coefficient was found to be .89 after one week[22].

Pros and cons of oophorectomy. Participants rated four reasons in favor of surgery and

seven reasons against surgery on a five- point scale (1=not at all, 5=completely) for how heavily each weighed in the woman's thinking about surgery. Participants were also asked to identify one factor each that was the most important weighing in favor of and against surgery. Items were developed based on the literature about issues related to PO and on women's reports about their concerns. In order to investigate the role of anxiety reduction as a motivation for surgery, we divided the pros scale into two components: anxiety-related and other benefits of surgery, creating composite scores for each component. There were two anxiety-related items ("Surgery would give me relief from fear of getting cancer" and "Having surgery would reduce the amount of uncertainty in my life") with a Cronbach's alpha of .89. The other four items ("I don't want to keep getting screening tests", "Ovarian cancer is difficult to detect early, when it is easier to treat", "I need to feel like I'm taking steps to prevent cancer" and "my physician recommended I have surgery) together have a Cronbach's alpha of .71. The cons showed low internal consistency (a=.41), reflecting that barriers varied from person to person. Thus, rather than create a composite score, we conducted the analyses using the individual barrier items (see Aiken et al., 1994 for a similar treatment of barriers to and benefits of mammography)[25].

Participants

Participants in the present study were women over the age of 25 with at least one first-degree relative (mother, sister, daughter) with ovarian cancer. As of January 1997, one hundred and seventy-seven women with at least one first degree relative with ovarian cancer had completed the educational component and one year follow-up. Of these 177 women, one had move out of the area and been terminated from the program, two had voluntarily opted out of the

study, one was deceased, and fifteen were excluded because they were participating in the Human Genome Project, leaving a total of 157 potential participants for the present study.

Of these 157, 6 could not be contacted because the address or phone number was out of date, and 39 were not be reached before the end of the study. Of the 111 women who were contacted by phone, three declined to participate. One woman was excluded because she stated that she had never heard of prophylactic surgery, and was unable to answer the questions in the interview. Thirteen of the women reached had already undergone prophylactic ovarian surgery.

A total of ninety-four women were interviewed for the present study. The mean age of participants was 40.27 years (s.d.=9.87). The majority of the participants were married (81.9%) and all but one were Caucasian. Three-quarters of the women had attended college or beyond. Twenty-five participants (26.6%) were classified as having a family history that consisted of sporadic cases of cancer. Forty-three women (45.7%) were classified as having a familial pattern, and 26 (27.7%) had a history consistent with a hereditary pattern.

RESULTS

Levels of current and future interest in PO

Forty-nine of the women in the study (52.1%) stated that they were not considering surgery at all at this time. One quarter of the sample (26.6%) were considering surgery somewhat, and just under one quarter were considering surgery at least moderately (see Table 1). A different picture emerges when the participants were asked about their interest in pursuing surgery in the future. Slightly less than one-third (31.9%) reported that it was not at all likely that they will undergo surgery. Over one third (35.1%) reported that they had some intention of having surgery, and one- third (32.9%) reported that their intentions were at least at a moderate level (see Table 1). None of the demographic variables were associated with current or future interest in surgery.

Table 1 about here

Of the 49 participants reporting that they were not currently considering prophylactic ovarian surgery, 20 stated that they had been considering surgery more strongly in the past. Half of those reporting that they had changed their mind had at one time been considering surgery very seriously.

Stress-related ideation and anxiety. The mean IES intrusive ideation score was 4.43 (s.d. = 6.64). Thirty-seven participants (40%) reported that they were experiencing no intrusive ideation at all. Thirty-nine (42%) reported low to moderate levels of intrusive ideation (scores of 1 to 9) and eleven (12%) reported moderate to severe symptoms (scores of 10-17). Six participants (6%) appeared highly distressed, with IES intrusion scores of 18 or greater. Because

of the large proportion of participants reporting no intrusive ideation, we dichotomized the scores into none vs. any intrusive ideation. Using this score, intrusive ideation was positively related to both current (X^2=6.4, p<.04) and future interest (X^2=6.16, p<.04) in surgery. Participants reported that when they do experience anxiety about the possibility of developing ovarian cancer, almost half become quite or extremely anxious. Only fifteen reported that they experience little or no anxiety. Cancer anxiety was not related to either current (r=.14, .n.s.) or future interest (r=.13, n.s.).

Perceived risk. Estimates of perceived risk ranged from 0 to 100, with a mean of 38.43 (s.d.25.05). Perceived risk was correlated with cancer anxiety (r=.22, p<.03) but only marginally with intrusive ideation (r=.18, p<.07). Perceived risk was positively associated with current interest in surgery (r=.28, p<.007) but not future interest (r=.19, n.s.)

Familial risk level and its influence on interest in prophylactic surgery and psychosocial variables. Interest in prophylactic surgery was spread evenly across the three objective risk groups. There was no difference among the three groups in terms of considering prophylactic surgery at present, [df4] χ^2 = 2.67, n.s. or likelihood of having surgery someday χ^2= 1.09, n.s. There was no difference between the three familial risk groups on intrusive ideation X^2 =1.73, n.s. or ovarian cancer anxiety F (2,91) =.288, n.s. Participants in the three objective risk groups did differ in perceived risk F (2,88) = 3.68, p<.03. Post-hoc analysis showed that women with a family history suggestive of inherited ovarian cancer risk perceived a greater risk to themselves (x=49.4, s.d.=25.5) than women with an apparently sporadic family history (x=32.0, s.d.=25.7) and a marginally greater risk than women with a familial risk pattern (x=35.7, s.d.=22.7).

(Table 2 about here)

Pros and cons of prophylactic surgery

After rating each item pro and con on a scale from one to five, participants were asked to name the factor that they felt weighed most heavily in favor of PO and the one that weighed most heavily against the procedure. The reason reported most frequently as the one that weighed most heavily in favor of PO was relief from fear of getting cancer (17%). Other items frequently reported as the most important were reducing the risk of ovarian cancer (16%), the difficulty of detecting ovarian cancer at an early, more treatable stage (14.9%), needing to feel like one was taking steps to prevent cancer (9.6%) and the physician's recommendation to undergo the procedure (4.3%). Seven out of 25 participants (28%) classified as having a sporadic family history ranked relief from fear/uncertainty as the most important reason in favor of surgery.

The reason reported most frequently as weighing the most heavily against surgery was the risk associated with undergoing surgery (23.7%). The next most frequently mentioned reason, that prophylactic surgery does not offer complete protection against cancer, was volunteered by nineteen of the women (20.4%) under "other." Had this been an item in the interview, the frequency might have been even higher. Other reasons given as the most important weighing against surgery were not being done with childbearing (6.5%), not wanting to go on hormone replacement therapy (6.5%), and low perceived risk of ovarian cancer (6.5%).

Anxiety/uncertainty reduction as a motivation for surgery was associated with current interest in PO, $F(2,91)=21.00$, $p<.0001$. Those who were very or somewhat interested in surgery were more likely to endorse anxiety/uncertainty reduction as a benefit of surgery than those who

were not interested. A similar relationship was observed between anxiety/uncertainty reduction and future interest in surgery, $F(2, 91) = 7.26$, $P<.001$. Other benefits of surgery were also associated with current interest in PO, $F(2, 91)=19.69$, $p<.0001$. Those not currently interested in surgery were less likely to rate benefits of surgery as weighing heavily in their decision than those who were somewhat or very interested. Benefits were also associated with future interest, $F(2,91)=16.20$. Those who were very interested in having PO someday were more likely to endorse benefits than those who were only somewhat interested, who in turn were more likely to endorse benefits than those who reported no future interest in PO.

The only reason against surgery that was associated with current interest in surgery was risk of surgery $F(2,91)= 3.42$, $p<.03$, such that those who were not interested in surgery were more likely to be concerned about risks of surgery than those who were very interested in surgery. The only reason against surgery associated with future interest in surgery was concern about exposure of risk status to one's insurance company or employer, $F(2,91)=5.41$, $p<.006$, such that those with a moderate future interest in surgery were more concerned about exposure of their risk status than those with no interest or strong interest.

Predictors of interest in prophylactic surgery

We used stepwise regression to examine predictors of current and future interest in surgery. For each outcome (current and future interest), the following independent variables were entered: family history, cancer anxiety, intrusive ideation, perceived risk, reducing anxiety/uncertainty, and other benefits of surgery. In addition, risk of surgery was added for current interest, and exposure of risk status was entered for future interest. The model for current

interest in surgery was significant, $F(3,87)=22.79$. Reducing anxiety/uncertainty was the strongest predictor of current interest, accounting for 28% of the variance. Other variables entering the equation were risk of surgery, and other benefits, accounting for an additional 9% and 5% of the variance respectively, for a total of 42%. The model for future interest in surgery was also significant, $F(1,89)=28.25$, $p<.0001$. Other benefits of surgery was the sole variable that entered the equation, accounting for 24% of the variance (see Table 3).

(Table 3 about here)

DISCUSSION

Among this sample of women with varying degrees of familial risk for ovarian cancer, half of the women were considering PO at least somewhat at the time of the study, and two-thirds did not rule out the possibility of undergoing the procedure in the future. Approximately 18% of the women reported moderate to high levels of intrusive thoughts about their risk level within the past week. Anxiety reduction was rated as the most important reason in favor of surgery by more women in the sample than any other reason in favor, including difficulty of detecting ovarian cancer early, needing to take steps to prevent cancer, reducing uncertainty and physician recommendation.

Among women who were currently considering PO, the desire to reduce anxiety and uncertainty was the strongest predictor of interest in the procedure, independent of family history or perceived risk. Indeed, a vital component of managing familial ovarian cancer risk, regardless of the decision one makes with regard to surgery, is the ability to manage one's anxiety about cancer over a long period of time, while adhering to a screening regimen that regularly exposes one to reminders of threat and uncertainty[17]. Research in other domains has shown that fixed attention on arousing stimuli can undermine efforts to persevere, whereas the ability to cool down the arousing properties of stimuli through abstraction when appropriate can promote adherence to tasks that require delayed gratification[17;26;27].

In the case of ovarian cancer risk, intrusive thoughts about the disease (which can include visual images, nightmares or physiological arousal at reminders) create a vivid focus on the threat of cancer, intensifying the desire for relief, which may override other factors that influence

decision-making about PO. The ability to cool off this process by shifting attention away from vivid images of risk to a more abstract consideration of the issues leads to a more informed, deliberate decision. We found that future interest in PO, which by definition is more abstract and removed from the heat of the present moment, was predicted by benefits such as low perceived efficacy of ovarian cancer screening and physician recommendation, but not by desire to reduce anxiety.

We also found both quantitative and anecdotal evidence that intrusive thoughts about risk and interest in PO fluctuated over time, and tend to increase in response to cancer-related cues. Almost half of the women in the study who were not currently considering prophylactic surgery at all reported that they had considered it in the past. Several of the women remarked during the interview that they were strongly considering surgery during or immediately after their relatives's illness or death, but that their interest in PO subsided over time. Recent exposure to reminders about one's cancer risk appeared to cause flare-ups in intrusive thoughts about the disease. For example, one woman was interviewed on the exact anniversary date of her mother's death due to ovarian cancer, and her IES score was extremely high. By contrast, another woman, an oncology nurse who was strongly considering surgery and who spoke at length about her general level of intrusive worry, had a low score on the IES. She attributed her responses to having been off from work for the past five days, and thus had not been exposed to any reminders. She stated that there have been times when she was caring for three ovarian cancer patients with advanced disease at once. At these times, she thought continuously about her personal risk level and about having surgery. Other milestones that may pose particular challenges for coping with anxiety

about one's of risk for ovarian cancer include reaching the age when a relative was diagnosed or deceased, and reaching menopause.

Exposure to cues will vary considerably both from person to person and over time. Some reminders will occur predictably (e.g., anniversary of mother's death, annual screening appointment) and some will be unpredictable (friend diagnosed with cancer). The fact that in the final regression equation for current interest in PO, desire to reduce anxiety/uncertainty was retained but that anxiety and intrusive ideation were not may reflect that some women who experience distressing thoughts about their risk may have effective strategies they can use other than surgery to cope with their distress. Identifying such strategies would aid in the design of interventions for individuals with a family history of cancer and would therefore be an important goal for future research.

The levels of interest in PO we found in this sample are comparable to those reported in previous studies[28;29], although unlike the participants in those studies, the women in our study were not from families in whom the presence of a BRCA1 mutation had been established. Genetic testing will increasingly become a useful tool in decision-making. Indeed, Struewing and colleagues found that of women who were interested in being tested for BRCA1 status, two-thirds of them cited decision-making about prophylactic oophorectomy as a motivation for undergoing genetic testing[29]. However, genetic test results can raise more questions than they answer. Many women who undergo genetic testing receive inconclusive results; that is, their family history is not accounted for by one of the known mutations. As genetic testing becomes widely available, and women with less striking pedigrees who would not have qualified for a

research protocol undergo testing, there will be increasing numbers of women who receive inconclusive results. The necessary ambiguity of risk information about ovarian cancer could lead anxious women to interpret information in the most threatening light. Anxiety produces an interpretive bias that skews people to think of something ambiguous as threatening,[30] even if the information is tentative good news (no known mutation detected).[31]

A limitation of the present study is that it was conducted with women attending a clinic for high-risk individuals, and may not represent women with a family history of ovarian cancer who do not seek out these services. However, the present sample does represent women who are educated about their risk level and who may present in doctors' offices with questions about prophylactic surgery. Another limitation of the present study is that the cross-sectional design does not show the process of adaptation to one's level of risk over time. A prospective study of changes in levels of interest in surgery over time would provide important information about how to manage counseling and informed consent procedures during these stressful junctures in the course of a lifetime of elevated risk by identifying the type and frequency of transition points when counseling about options would be needed the most.

Educational interventions that merely present factual information about familial risk of cancer may be insufficient to address anxiety and uncertainty about one's risk level. Lerman and colleagues[21] have found that the tendency to overestimate one's risk of breast cancer in this fashion can persist even after receiving a personalized risk counseling session. High levels of anxiety and stress-related ideation have been shown to interfere with ability to recall threat-related information. If a woman is distressed about her risk for cancer to the extent that she cannot recall

important information related to her decision, her desire for immediate relieve may lead her to not fully consider the implications of undergoing PO, such as the need for ongoing surveillance and for consideration of HRT. In this manner, the presence of persistent intrusive worries about cancer risk can undermine one's ability to give informed consent. Fortunately, empirical studies have demonstrated that effective, short-term therapy exists for the management of anxiety and intrusive ideation. These modalities include exposure therapy, systematic desensitization, and stress inoculation training[32;33].

Targeted psychological interventions for cancer anxiety constitute a minimally invasive option for women whose sole indication for prophylactic surgery is fear of cancer, and may enhance full consideration of post-surgical implications for those with other indications for surgery. Such an intervention would include desensitization, education about the fluctuating nature of intrusive thoughts about one's risk, and cognitive-behavioral strategies for coping with anxiety triggers such as anniversary of family member's death. After undergoing such an intervention, if a woman still wanted to undergo PO, she would be in a better position to give informed consent. Anxiety management may also benefit those who are postponing PO until they have completed childbearing, to help them cope with their worries while waiting to undergo the procedure. Expanded informed consent protocols for prophylactic surgery that incorporate choices about treatment modalities for anxiety would allow women to make risk management decisions that take both their long-term physical and emotional well-being into account.

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Table 1. Frequencies of current and future interest in prophylactic oophorectomy.

	Current interest in PO	Future interest in PO
Not at all	49 (52.1%)	30 (31.9%)
Somewhat	25 (26.6%)	33 (35.1%)
Moderately	9 (9.6%)	16 (17.0%)
Strongly	9 (9.6%)	10 (10.6%)
Very strongly	2 (2.1%)	5 (5.3%)

Table 2. Factors associated with current and future interest in PO

	Current interest in PO	Future interest in PO
Objective risk classification	n.s.	n.s.
Perceived risk	$F(2,91)=3.84$, $p<.02$	n.s.
Intrusive ideation	$X^2=6.4$, $p<.04$	$X^2=6.16$, $p<.04$
Ovarian cancer anxiety	n.s.	n.s.
Anxiety/uncertainty reduction	$F(2,91)=21.00$, $p<.0001$.	$F(2,91) = 7.26$, $P<.001$.
Benefits of surgery	$F(2,91)=19.69$, $p<.0001$	$F(2,91)=16.20$, $p<.0001$
Risks of surgery	$F(2,91)$ 3.42, $p<.03$	n.s.
Concern about privacy	n.s.	$F(2,91)=5.41$, $p<.006$

Table 3. Stepwise regression equations predicting levels of interest in PO

Current Interest in PO

Variable	r^2
Reducing anxiety/uncertainty	.28
Risks of surgery	.09
Benefits	.05

Future Interest in PO

Variable	r^2
Benefits	.24

APPENDIX – PROJECT III

APPENDIX A

CASE REPORT FORMS

98-029

Case Report Form Checklist

Participant Initials:_____ **MR#**_____ **Study ID#**_____

Date of Oophorectomy:_____

	Tissue Donation Only	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Post Surgery
Date									
Signed consent: Approval Date:_____									
Eligibility Checklist									
On Study Form									
Past Medical Hx									
Surgical Hx									
Cancer Related Hx									
Family Hx of Cancer (Source Doc)									
Social Hx (Source Doc)									
Gyn Past Hx									
Gyn Exam									
Dietary Assessment (Source Doc)									
HHQ (Source Doc)									
Pre-Tx signs/symptoms									
Labs									
Lab (Source Doc)									
Participant Questionnaire									
Con Meds									
Tox/Med Problems									
Compliance Measures									
Pill Calendar (Dates)									
PE									
PE (Source Doc)									
Study Drug Administration									
Oophorectomy Surgical Notes (Source Doc)									
Surgical Pathology (Source Doc)									
Off Study Form									
Patient Telephone Contact (as needed)									
Reviewed/ Date									

Recruitment Form

A Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

Study ID#_____ **Medical Record#**_____ **Initial Screening Date:**_____

Name: _____

Address: _____

City, State _____

Zip Code: _____

Phone# **Home:**_____ **Work:**_____

Date of Birth: _____ **Age:**_____

Must be 18 years of age or older

Source Documentation:

Where did you learn about this study? _____

Eligibility Determination: ***both questions must be answered "YES"***

(1) Has determined with her physician that prophylactic oophorectomy (surgical removal of ovaries) may be advisable to possibly prevent ovarian cancer.	**Yes No**
(2) Has undergone genetic risk counseling regarding her possible increased risk for ovarian cancer. If no, refer to FRAP. Joanne Spoltore @ (215)-728-2795 or 1-800-325-4145	**Yes No**

FRAP ID#_____ ***Re-Screen Date:***_____

Purpose:

This is a study to determine important information about the drug fenretinide to decide its potential use as a cancer preventive drug. The purpose of this research study is to learn why some ovaries develop cancers, and whether a new investigational agent fenretinide, will stop cancer processes in the ovary. Interested women who plan to have their ovaries removed to prevent cancer will complete questionnaires and have a physical examination along with blood tests to make sure they are in good medical condition. Participants will be randomized to treatment with either placebo or fenretinide. For approximately 4 months they will take 4 tablets daily except for 3 days of each month. During this time period they will be watched closely. At the end of the 4-6 month treatment period, they will undergo their planned prophylactic oopherectomy. At that time, they will allow their ovaries after surgical removal to be used for research purposes.

Interest:

Are you interested in making an appointment with a doctor to discuss your participation in this study? **Yes No**

If not interested, why? _____

Appointment:

Date:_____ **Time:**_____

Interviewer:_____

A PHASE II EVALUATION OF FENRETINIDE (4-HPR)
AS A CHEMOPREVENTIVE AGENT FOR OVARIAN CARCINOMA

IRB 98-029

ON-STUDY FORM

MR#_____ NAME_____ _____ _____
Last First MI

DATE OF BIRTH:____/____/____ RACE: _____ SEX: FEMALE

PROTOCOL #: IRB 98-029 STUDY ID# _____ BASELINE PS: _____

DATE OF SIGNED CONSENT FOR FULL STUDY: ____/____/____ DATE OF FIRST STUDY DRUG: ____/____/____

DATE OF SIGNED CONSENT FOR TISSUE DONATION ONLY: ____/____/____

TREATING MD: _____ HOSPITAL: _____

Entry into PTS:

Entry into Database:

A Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma
IRB 98-029
ELIGIBILITY CHECKLIST

Study ID#_____ Participant Initials:_____ Medical Record#_____

Reasons for Eligibility (All responses must be YES)

YES	NO	
		Female ≥ 18 years of age.
		Has decided to undergo prophylactic oophorectomy because of increased risk for ovarian cancer. ( ) Increased risk for ovarian cancer secondary to evidence of a genetic defect (BRCA1 or BRCA2). ( ) Increased risk for ovarian carcinoma secondary to a family history of one or more 1st degree relatives diagnosed with ovarian cancer prior to the age of 50 years. ( ) Increased risk for ovarian cancer secondary to a family history of • one 1st degree relative diagnosed with ovarian cancer (any age) *and* • one or more 1st or 2nd degree relative diagnosed with breast or ovarian cancer (any age).
		Has agreed to schedule prophylactic surgery in 4-6 months.
		Has ECOG performance status of 0-1 and a life expectancy of at least 12 months. **PS=____**
		Date of Labs.=_____ Must be performed within 28 days prior to study treatment. Does participant have : ( ) adequate bone marrow function: WBC ≥ 4,000 µL and platelet count ≥ 100,000 µL **WBC=_____ PLT=_____** ( ) adequate liver function: Bilirubin < 1.5 mg/100 ml and SGOT < 2X normal **Bilirubin=_____ SGOT=_____** ( ) adequate renal function: Creatinine < 1.5 mg/100 ml or Creatinine Clearance (60 µl/min.) **Creatinine=_____ and/or Creatinine Clearance=_____** ( ) Fasting Triglyceride less than twice the upper limit of normal range. **Fasting Triglyceride=_____** ( ) Negative serum pregnancy test performed within 7 days of initiating the study drug. **Date of serum pregnancy test:_____**
		Has had a normal pelvic exam within 6 weeks prior to study drug administration. **Date of pelvic exam=_____**
		Has the participant signed an informed consent indicating that they are aware of the investigational nature of this study? **Date of informed signed consent:_____**

Reasons for Ineligibility (All responses must be NO)

YES	NO	
		Does the participant have any major cardiac, respiratory, neurologic or psychiatric disabilities?
		Has the participant received recent (within 5 years) chemotherapy, radiotherapy or other investigational agents (within 6 months).
		Does the participant have concurrent malignancies or any prior cancer history within 5 years except non-melanomatous skin cancers?
		Is the participant pregnant or lactating?
		If the participant is of childbearing potential, is she unwilling to use barrier contraceptive methods during the study time period?
		Is participant currently using oral, injectable or implanted contraceptives? Participant cannot have used oral, injectable or implanted contraceptives for three (3) months prior to the start of this study.
		Is the participant taking vitamin A supplements other than that amount contained within the over-the-counter multivitamin? (Supplement must contain less than 25,000 IU of vitamin A.)
		Is participant currently taking hormone replacement therapy or has she taken hormone replacement therapy within the last 6 months?
		Has the participant used non-steroidal anti-inflammatory drugs (NSAID's) greater than 3 times per month in each of the 6 months prior to study entry?

Physician Signature:_____ Date:_____

Entry into Database:

A Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

IRB 98-029

ELIGIBILITY CHECKLIST FOR TISSUE DONATION ONLY

Study ID#_____ **Participant Initials:**_____ **Medical Record#**_____

Reasons for Eligibility (All responses must be YES)

YES	NO	
		Female ≥ 18 years of age.
		Has decided to undergo prophylactic oophorectomy because of increased risk for ovarian cancer. ( ) Increased risk for ovarian cancer secondary to evidence of a genetic defect (BRCA1 or BRCA2). ( ) Increased risk for ovarian carcinoma secondary to a family history of one or more 1st degree relatives diagnosed with ovarian cancer prior to the age of 50 years. ( ) Increased risk for ovarian cancer secondary to a family history of • one 1st degree relative diagnosed with ovarian cancer (any age) *and* • one or more 1st or 2nd degree relative diagnosed with breast or ovarian cancer (any age).
		Has the participant signed an informed consent indicating that after their planned oopherectomy they are willing to donate their ovarian tissue to research. **Date of informed signed consent for tissue donation:**

Physician Signature:_____ ***Date:***_____

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06/14/99

Entry into Database:

Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

IRB 98-029

PAST MEDICAL HISTORY

Study ID #:_____ Participant Initials:_____ MR#:_____

PLEASE PROVIDE A RESPONSE :	NO	YES	IF YES IS CHECKED, PROVIDE DETAILS
HEENT DISEASE:			
CARDIOVASCULAR DISEASE:			
BRONCHOPULMONARY DISEASE:			
HEPATOBILIARY DISEASE:			
GASTROINTESTINAL DISEASE:			
GENITOURINARY DISEASE:			
ENDOCRINE/METABOLIC DISORDERS:			
HEMATOLOGICAL DISEASE:			
DERMATOLOGICAL DISEASE:			
MUSCULOSKELETAL DISEASE:			
NEUROLOGICAL DISEASE:			
PSYCHOLOGICAL DISEASE:			
IMMUNOLOGICAL DISEASE:			
ALLERGY:			
SURGICAL HISTORY:			If yes, complete- "Surgical History" Form
CANCER HISTORY:			If yes, complete - "Cancer Related Treatment" Form
OTHER: (specify)			

*Physician/Coordinator Signature:*_____ *Date:*_____

Entry into Database:

A Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

IRB 98-029

GYN PAST HISTORY

Study ID# _____ Participant Initials: _____ Medical Record# _____

	Yes	No
How old were you when your periods began?		____age
Do you currently have menstrual periods?		
Is your cycle regular?		
If regular, what is your average cycle length?		____days
Date of last menstrual period.		/ /
If you are not menstruating, how old were you when you stopped?		____age
- If you are not menstruating, why did you periods stop?		
Natural menopause		
Surgical hysterectomy		
Other:		
Have you been pregnant?		
- If yes, how many pregnancies?		____#
- How old were you when you had your first born?		____age
- How many live children?		____#
- How many miscarriages / abortions?		____#
Have you ever used birth control pills?		
- If yes, for how many years?		____# years
- What types? _____ _____ _____		Date Stopped: _____ _____ _____
- Are you currently using any other birth control method?		
Methods:		
- intrauterine device (IUD)		
- barrier method contraceptive (condom or diaphragm)		
- injectable or implanted contraceptive		
If yes, for how many years?		____# years Date Stopped: / /
Have you ever used hormone replacement medication?		
- If yes, for how many years?		____# years Date Stopped: / /
Have you ever had		
- Endometriosis		
- abnormal PAP smear		
- Pelvic Inflammatory Disease		
- Ovarian Cysts		
Have you ever used products which contain talc? (e.g. dusting powder with talc)		
Have you ever taken a drug for infertility (to try and become pregnant) or because your periods stopped?		
- Clomid _____# of years Date Stopped:_____		
- Pergonal _____# of years Date Stopped:_____		
- Serophene _____# of years Date Stopped:_____		
- HCG _____# of years Date Stopped:_____		
- Other: specify_____ _____# of years Date Stopped:_____		

Physician / Coordinator Signature: _____ Date: _____

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06/14/99 ; 08/17/99

Entry into Database:

A Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

IRB 98-029

SURGICAL HISTORY

Study ID#_____ **Participant Initials:**_____ **MR#**_____

Previous Surgery: (If none, circle none) **NONE**

Procedure / Site	Date

Physician/ Coordinator Signature:_____ ***Date:***_____

Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

IRB 98-029

CANCER RELATED TREATMENT

Study ID#____________ Participant Initials:______________ MR#______________

Please provide the following information for each previous cancer diagnosis.

Diagnosis	*Date of Diagnosis*	*Stage*
Location	*Cell Type*	*Surgical Procedure*
Immunotherapy/Hormone Therapy	*Chemotherapy*	*Radiation Therapy*

Diagnosis	*Date of Diagnosis*	*Stage*
Location	*Cell Type*	*Surgical Procedure*
Immunotherapy/Hormone Therapy	*Chemotherapy*	*Radiation Therapy*

Diagnosis	*Date of Diagnosis*	*Stage*
Location	*Cell Type*	*Surgical Procedure*
Immunotherapy/Hormone Therapy	*Chemotherapy*	*Radiation Therapy*

Physician / Coordinator Signature:____________________ Date:__________

Prevention Clinical Trials: version 01/21/99 tch 06/14/99

Entry into Database:

Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma
IRB

CONCOMITANT MEDICATIONS

Study Id#________ Participant Initials:__________ Medical Record#____________

Concomitant Medication	Baseline / /	Visit Date / /	Visit Date / /	Visit Date / /	Visit Date / /	Visit Date / /
Med:	Pre-Study ☐	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:
Dose:	Or	or	or	or	or	or
Schedule:	Start Date	Stop Date	Stop Date	Stop Date	Stop Date	Stop Date
Reason:	/ /	/ /	/ /	/ /	/ /	/ /
Med:	Pre-Study ☐	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:
Dose:	or	or	or	or	or	or
Schedule:	Start Date	Stop Date	Stop Date	Stop Date	Stop Date	Stop Date
Reason:	/ /	/ /	/ /	/ /	/ /	/ /
Med:	Pre-Study ☐	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:
Dose:	or	or	or	or	or	or
Schedule:	Start Date	Stop Date	Stop Date	Stop Date	Stop Date	Stop Date
Reason:	/ /	/ /	/ /	/ /	/ /	/ /
Med:	Pre-Study ☐	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:
Dose:	or	or	or	or	or	or
Schedule:	Start Date	Stop Date	Stop Date	Stop Date	Stop Date	Stop Date
Reason:	/ /	/ /	/ /	/ /	/ /	/ /
Med:	Pre-Study ☐	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:
Dose:	or	or	or	or	or	or
Schedule:	Start Date	Stop Date	Stop Date	Stop Date	Stop Date	Stop Date
Reason:	/ /	/ /	/ /	/ /	/ /	/ /
Med:	Pre-Study ☐	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:	Ongoing ☐ Start Date:
Dose:	or	or	or	or	or	or
Schedule:	Start Date	Stop Date	Stop Date	Stop Date	Stop Date	Stop Date
Reason:	/ /	/ /	/ /	/ /	/ /	/ /
Physician/Coordinator Initial						
Entry into Database: Initial/Date						

Prevention Clinical Trials:version: 06/14/99 tch

Currently Used Medications, Vitamins, and Supplements

Participant Name_____ **Medical Record #**_____

Protocol # 98-029 **Study ID #** _____

Prescription Drugs

Drug	*Strength*	*# of times you take per day*	*Reason Used*	*Date Started*

Over the Counter Medications (e.g., Aspirin, Tylenol, Benadryl, Contac, Pepcid AC, etc.)

Medication	*Strength*	*# of times you take per day*	*Reason Used*	*Date Started*

Vitamin Supplements (include multivitamins, individual vitamins, etc.)

Vitamin	*Strength*	*# of times you take per day*	*Reason Used*	*Date Started*
Within Multivitamin: Vitamin A				
Vitamin C				
Vitamin E				

Nutritional Supplements (e.g., Cod Liver Oil, Calcium, etc.)

Supplement	*Strength*	*# of times you take per day*	*Reason Used*	*Date Started*

Participant Signature:_____ ***Date:*** _____

Reviewed by Physician/Study Coordinator:_____ ***Date:***_____

PARTICIPANT QUESTIONNAIRE
IRB 98-029

Study ID#_____ Participant Name:_____ Date: ___/___/___ to ___/___/___

The following questionnaire is being given to you to help us determine any problems or side effects that you may have experienced during your participation on a Prevention Trial. Please complete the following statements by circling "yes" or "no". If the answer is "yes", circle the number that best describes the severity. Also, mark down the dates that it occurred and any comments that you feel are important.

SEVERITY:
0= None Present 1= Mild 2= Moderate 3= Severe

PLEASE BRING THIS QUESTIONNAIRE TO EACH APPOINTMENT.

PROBLEM	Since Last Visit?		Severity	Dates that it occurred	Comments
Trouble seeing at night	Yes	No	0 1 2 3		
Do you adapt easily from light to semi-darkness?	Yes	No	0 1 2 3		
In a semi-dark environment, can you perceive the outlines of objects?	Yes	No	0 1 2 3		
When you pass from a semi-dark environment to a strongly lit one, does your vision recover quickly?	Yes	No	0 1 2 3		
Nausea	Yes	No	0 1 2 3		
Itching	Yes	No	0 1 2 3		
Skin Rash	Yes	No	0 1 2 3		
Dry Eyes	Yes	No	0 1 2 3		
Dry Nose	Yes	No	0 1 2 3		
Dry Lips	Yes	No	0 1 2 3		
Muscle Aches	Yes	No	0 1 2 3		
Fatigue	Yes	No	0 1 2 3		
Hair Loss	Yes	No	0 1 2 3		
Sensitivity of eyes to sunlight	Yes	No	0 1 2 3		
Eye Irritation	Yes	No	0 1 2 3		
Headaches	Yes	No	0 1 2 3		
Dizziness	Yes	No	0 1 2 3		
Vertigo (sensation of the room spinning)	Yes	No	0 1 2 3		
Fatigue	Yes	No	0 1 2 3		
Muscles Aches	Yes	No	0 1 2 3		
Bone/joint pain	Yes	No	0 1 2 3		
Indigestion	Yes	No	0 1 2 3		
Vomiting	Yes	No	0 1 2 3		
Diarrhea	Yes	No	0 1 2 3		
Abdominal Pain	Yes	No	0 1 2 3		
Other	Yes	No	0 1 2 3		

Participant Signature:_____ Date:_____

Reviewed by Physician / Coordinator:_____ Date:_____

GENERAL HEALTH QUESTIONS

Answer each question by placing an "X" on the line provided.

1. **In general, would you say your health is:**

   ____Excellent ____Very Good ____Good ____Fair ____Poor

2. **Compared to your last visit, how would you rate your health now?**

   ____ Much better now than the last visit

   ____ Somewhat better now than the last visit

   ____ About the same as the last visit

   ____ Somewhat worse now than the last visit

   ____ Much worse now than the last visit

3. **The following statements are about common activities you may perform on a typical day. Does your health limit you in these activities? If so, how much? Please an "X" in the appropriate box.**

ACTIVITY	Yes, Limited a Lot	Yes, Limited a Little	No, Not Limited at all
Vigorous Activities, such as running, lifting heavy objects, strenuous sports			
Moderate Activities, such as moving a table, vacuuming, bowling, golfing			
Lifting or carrying groceries			
Climbing *several* flight of stairs			
Climbing *one* flight of stairs			
Bending, kneeling, stooping			
Walking *more than a mile*			
Walking *several blocks*			
Walking *one block*			
Bathing or dressing yourself			

4. **Since your last visit, have you had any of the following problems with your work or other regular daily activities as a result of your physical health? Place an "X" in the appropriate box.**

	Yes	No
Cut down the amount of time you spend on work or other activities		
Accomplished less than you would like		
Were limited in the kind of work or other activities		
Had difficulty performing the work or other activities (example, it took extra effort)		

Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

IRB 98-029

PHYSICAL EXAMINATION

Study ID#_____ Participant Initials_____ MR#_____

Date of Examination	Blood Pressure	Pulse	Respirations	Temp °F	Performance Status	Height* inches	Weight* lb.	BSA*
	/				0 1 2 3 4			

** Required only on Initial Physical Exam*

Month# _____	Circle: 1= Normal 2=Abnormal 3=Not Done			Specify if Abnormal
HEENT	1	2	3	
SKIN	1	2	3	
NODES	1	2	3	
HEART	1	2	3	
LUNGS	1	2	3	
BREAST	1	2	3	
ABDOMINAL	1	2	3	
GU/RECTAL	1	2	3	
BACK	1	2	3	
EXTREMITIES	1	2	3	
NEURO	1	2	3	
OTHER	1	2	3	

Physician Signature:_____ Date:_____

A Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

98-029

GYN PHYSICAL EXAMINATION

Study ID#_____ **Participant Initials:**_____ **Medical Record#**_____

Date of Examination _____/_____/_____ **Month:** *_____

******Baseline:* **must have had a normal pelvic exam within 6 weeks of study drug administration.**

	Circle: 1=Normal 2=Abnormal 3=Not Done	Specify if Abnormal
Speculum Exam		
- vaginal cylinder	1 2 3	
- cervix	1 2 3	
- other	1 2 3	
Bi-manual Exam	1 2 3	
Rectovaginal Exam	1 2 3	
External Genitalia	1 2 3	
Inguinal Nodes	1 2 3	
	1 2 3	
	1 2 3	

Physician Signature:_____ **Date:**_____

Prevention Clinical Trials: version 01/21/99 tch

Entry into Database:

Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

IRB 98-029

PRE-TREATMENT SIGNS AND SYMPTOMS

Study ID#_____ **Participant Initials**_____ **MR#**_____

List all pre-treatment signs and symptoms. (If none, circle none).

Pre-Treatment Signs and Symptoms	NCI CTC Grade 1= Grade 1 2= Grade 2 3= Grade 3 4= Grade 4	Treatment Required 1= None 2= OTC 3= Prescription Drug 4= Hospitalization 5= Non-Drug Therapy
NONE		

Physician / Coordinator Signature:_____ **Date:**_____

Entry into Database:

Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

IRB 98-029

STUDY DRUG ADMINISTRATION

Study ID#_____ **Participant Initials:**_____ **MR#**_____

Month	Route	Dosage (mg)	Start Date / /	Stop Date / /	Entry into Database (Initials/date)
	PO				
	PO				
	PO				
	PO				
	PO				
	PO				
	PO				
	PO				

COMMENTS:

_____

_____

_____

_____

_____

FOX CHASE CANCER CENTER
IRB 98-029
PILL CALENDAR RECORD

Participant Name:_____ **Medical Record #** _____

Study ID # _____ **Date:**_____/_____/_____ **to** _____/_____/_____

You will take the tablets for 28 days in a row, then not take any tablets for 3 days. It is important to take your tablets at the same time each day with a meal. Record the number of tablets you take each day on the calendar below. If you miss a day, *DO NOT* take the missed dose on a different day. Record the missed day on the calendar with a zero.

If you develop any side effects from the tablets, record the information on the Participant Questionnaire.

If applicable, please record each day you menstruate with an "M".

Contact Dr. Christine Szarka at (215) 728-4300 or Cecilia McAleer at (215) 728-2981 if you have any serious problems or concerns.

Bring this calendar and your pill bottle with you each time you have an appointment.

YOUR NEXT APPOINTMENT IS_____/_____/_____ **at**_____ **with Dr.**_____

Sunday	*Monday*	*Tuesday*	*Wednesday*	*Thursday*	*Friday*	*Saturday*
☐	☐	☐	☐	☐	☐	☐
☐	☐	☐	☐	☐	☐	☐
☐	☐	☐	☐	☐	☐	☐
☐	☐	☐	☐	☐	☐	☐
☐	☐	☐	☐	☐	☐	☐

A Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

IRB 98-029

PATIENT TELEPHONE CONTACT DOCUMENTATION FORM

Study ID#_____ Participant Initials:_____ Medical Record#_____

	Contact # 1 ___/___/___	*Contact # 2* ___/___/___	*Contact # 3* ___/___/___	*Contact # 4* ___/___/___	*Contact # 5* ___/___/___
Is participant still taking Fenretinide?					
How compliant is the participant? • **any missed doses?** • **reasons?**					
Does the participant have any toxicities from the Fenretinide?					
Comments: • **Recommendations made to participant** • **Other medications patient is using** • **Other pertinent information**					
Reviewed by: Coordinator/Date					

Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

IRB 98-029

COMPLIANCE MEASURES

Study ID#_____ Participant Initials:_____ MR#_____

Provide the following information regarding the participant's compliance with pill taking and ability to keep appointments.

1. **Date Drug Started** __/__/__
2. **Date Drug Ended** __/__/__
3. **Number of Pills (Days) Patient Should Have Taken** _____
4. **Number of Pills Taken** _____
5. **% of Prescribed Pills Taken** _____

Level of Adherence	*% Pills Taken*	*% Calendar Completed*	*Appointment Kept/Missed*
I	85-100 %	83-100 %	Kept as appointed
II	75-84 %	66-82 %	Kept within 14 days
III	65-74 %	25-65 %	Kept within 15-30 days
IV	< 65 %	< 25 %	Kept within > 30 days
V	None	None	None
SCORE	**P=**	**C=**	**A=**

Participant is considered compliant if level of adherence is I or II for each category.

Prevention Clinical Trials version:01/21/99 tch

Entry into Database:

Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

IRB 98-029

TOXICITIES / MEDICAL PROBLEMS

Study ID#_____ Participants Initials:_____ Medical Record#_____

Date:____/____/____ Month:_____

NCI CTC GRADE	STUDY DRUG RELATIONSHIP	ACTION TAKEN REGARDING STUDY DRUG	TREATMENT REQUIRED	PATIENT OUTCOME
1= Grade 1	1= Not Related	1= None	1= None	1= Recovered
2= Grade 2	2= Unlikely Related	2=Study Drug Dosage Changed	2= OTC	2= Medical Problem Still Present
3= Grade 3	3= Possibly Related	3= Study Drug Temporarily Changed	3= Prescription Drug	3= Alive with Sequelae
4= Grade 4	4= Probably Related	4=Patient Off Study due to this problem	4= Hospitalization	4= Death
	5= Related		5= Non-Drug Therapy	

List any new or continuing signs, symptoms or medical problems(s) since last visit.
If NONE, circle NONE:

Toxicity	Onset Date / End Date	Grade	Study Drug Relationship	Action Taken	Treatment Required	Patient Outcome
NONE						

Physician / Coordinator Signature:_____ Date:_____

Entry into Database:

Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

IRB 98-029

LABORATORY DATA

Study ID#_____ Participant Initials_____ MR#_____

	Baseline * / /	/ /	/ /	/ /
WBC				
Hgb				
Hct				
Plts				
Neutro /Bands %				
Lymphocytes %				
Monocytes %				
Eosinophils %				
Basophils %				
Sodium				
Potassium				
Chloride				
Bicarbonate				
Glucose				
BUN				
Creatinine				
Alk. Phos.				
LDH				
Total Bilirubin				
SGOT (AST)				
SGPT (ALT)				
Fasting Lipid Profile				
Cholesterol				
Triglycerides				
HDL				
LDL				
VLDL				
Serum Pregnancy Test				
Entry into Database Initials/Date				

*** Baseline: to be performed within 28 days prior to study treatment.**

A Phase II Evaluation of Fenretinide (4-HPR) as a Chemopreventive Agent for Ovarian Carcinoma

IRB 98-029

OFF STUDY

Study ID#_____ Participant Initials_____ MR#_____

Date of Oopherectomy:_____
Physician:_____
Hospital:_____

Date of Menstrual Cycle:_____

Date Off Study:_____

Reason: (Check the primary reason for study termination)

Study completed	_____	
Refused further treatment	_____	
Toxicity	_____	
Protocol Violation	_____	
Other Medical Problems	_____	
Other	_____	Explain:_____
*Death	_____	

*If patient died while on study:
Date of Death _____
Was an autopsy performed: (___)No (___)Yes
Cause of Death:_____

Additional Comments:

_____

_____

_____

_____

Prevention Clinical Trials: version 01/21/99 tch
06/14/99

Entry into Database:
Entry into PTS: