

ASCUS-LSIL Triage Study

Design, Methods and Characteristics of Trial Participants

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OBJECTIVE: To describe the design and methods of the ASCUS-LSIL Triage Study (ALTS), a multicenter, randomized clinical trial designed to evaluate three alternative methods of managing low grade (LSIL) and equivocal (ASCUS) cervical cytologic diagnoses.

STUDY DESIGN: Non-pregnant women, 18+ years old, with ASCUS or LSIL, no prior hysterectomy or ablative therapy to the cervix, were referred to one of four clinical centers around the United States. Eligible and consenting participants were administered a risk-factor questionnaire and underwent a pelvic examination, collection of cervical specimens for liquid-based cytology and human papillomavirus (HPV) testing and Cervicography™ (National Testing Laboratories, Fenton, Missouri, U.S.A.). Patients were randomized to one of three

arms: (1) immediate referral for colposcopy at enrollment, (2) follow-up with cytology only, and (3) use of HPV DNA testing to triage to colposcopy. All women are followed every six months for two years with pelvic examinations, cytologic and masked HPV testing, and masked Cervicography™. Digital cervical images and cytology and histology slides are externally reviewed to maximize patient safety.

ALTS is a demanding, collective effort that will prove its worth only if the results are clear and valuable to both clinicians and researchers.

RESULTS: We enrolled and randomized 3,488 eligible women with ASCUS and 1,572 women with LSIL.
CONCLUSION: The successful enrollment, randomization and high rates of follow-up are encouraging. The study will help clarify the optimal strategies for managing low grade cervical abnormalities. (*Acta Cytol* 2000;44:726-742)

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There is controversy in the United States over the proper evaluation and management of low grade (LSIL) and equivocal (ASCUS) cervical cytologic diagnoses.^{3,10,17,19,21,26,30} To address this problem, the National Cancer Institute initiated the ASCUS-LSIL Triage Study (ALTS). ALTS is a multicenter, randomized clinical trial designed to evaluate three alternative methods of management: immediate colposcopy, cytologic follow-up and triage by human papillomavirus (HPV) DNA testing. This paper describes the design and methods of the trial as well as the demographic and clinical characteristics of the 5,060 women randomized by the end of enrollment in December 1998.

In the United States, The Bethesda System^{3,28} has replaced the Papanicolaou (Pap) classification for reporting the results of cervical/vaginal cytology. This system is concordant with recognition that HPV infection causes virtually all cases of cervical carcinoma and its precursor intraepithelial lesions.^{2,16} Within the Bethesda System, intraepithelial squamous cervical abnormalities fall into three categories²²:

- HSIL (high grade squamous intraepithelial lesion) includes cervical intraepithelial neoplasia 2 (CIN 2), also known as moderate dysplasia, and CIN 3, which includes severe dysplasia and carcinoma *in situ*. These lesions, especially CIN 3 (carcinoma *in situ*), are the known precursors of invasive carcinoma in that they represent virtually complete replacement of the cervical epithelium with neoplastic cells.

- LSIL (low grade squamous intraepithelial lesion) represents cytologic evidence of HPV infection. It includes cervical intraepithelial neoplasia 1 (CIN 1), also known as mild dysplasia, and the HPV-associated diagnoses that were previously included as part of class 2 of the Pap classification. The characteristic changes of HPV infection, which are often but not always seen in LSIL, are a particular kind of nuclear wrinkling and perinuclear cytoplasmic cavitation, described as either koilocytotic or condylomatous atypia.

- ASCUS (atypical squamous cells of undetermined significance) includes cellular changes that do not fulfill the criteria for low or high grade squamous intraepithelial lesions. ASCUS subsumes some minor abnormalities commonly grouped as "atypical" in class 2 of the Pap classification, but be-

nign changes are excluded. The "undetermined" significance reflects both the lack of sufficient morphologic features to allow a definitive diagnosis and the uncertain relation of these cells to infection with HPV.

Globally, cervical cancer is the second or third most common malignancy in women.³² In the United

The large number of enrolled women, successful randomization and high rates of follow-up are very encouraging.

States, there are about 12,800 cases of carcinoma, resulting in about 4,600 deaths annually.¹³ Approximately 0.6%, or 300,000, of the 50 million Pap smears estimated to be performed each year are diagnosed as HSIL.⁷ In comparison, about 2–3% of smears obtained are diagnosed as LSIL. The prevalence of LSIL is a function of the prevalence of acute, sexually transmitted HPV infection, which, in turn, is highly dependent on the age and related numbers of new sexual partners of the population being screened.³⁷ The prevalence of ASCUS is more arbitrary because it represents a poorly defined diagnostic fraction of the previous, extensive, "benign atypia," or class 2 classification.⁴⁰ In the United States, over 2 million Pap smears per year are diagnosed as ASCUS.¹³

Virtually all U.S. health care providers agree that women with cytologic HSIL require colposcopic examination and that those with colposcopic evidence of HSIL require cervical biopsy. If the histologic diagnosis is CIN 2 or more severe, ablative or excisional treatment now relies most commonly on the electrosurgical loop excision procedure (LEEP), cryosurgery or, more rarely, cold-knife conization. No universal agreement exists for managing LSIL and ASCUS. Most low grade lesions will regress spontaneously,²¹ and many equivocal lesions will be shown to be benign. However, management of ASCUS/LSIL is potentially of concern given that a small but important minority may have HSIL or even carcinoma upon colposcopy and biopsy.^{10,31,42} As a result, many clinicians are not willing to follow ASCUS/LSIL for possible regression out of concern for missing underdiagnosed HSIL. Unreasonable societal expectations of perfect cervical cancer prevention have increased the possibility of

litigation whenever a false negative screen or undertreatment occurs.

Current management often includes colposcopically directed biopsy to confirm the severity of the disease and cervical ablation or excision of even low grade or equivocal lesions (and the cervical transformation zone) to prevent progression. This has led to an increased burden on already-limited colposcopy services. The cost of these services and subsequent overtreatment is considerable. Medical complications of treatment are rare but include cervical incompetence, secondary infertility, infection and cervical stenosis.⁸ Furthermore, emotional concerns regarding referral and treatment for persistent viral infections and "precancerous conditions" are sometimes substantial.³⁴

The need for clear management guidelines for women with ASCUS and LSIL—specifically, for more cost effective approaches to triaging women to colposcopy—was discussed at the Bethesda 2 Workshop, conducted in 1991. This was followed by a National Cancer Institute (NCI)-sponsored workshop to discuss the feasibility of conducting a randomized clinical trial and to outline the viable management strategies to be evaluated. Although the participants disagreed strongly as to the proper management of ASCUS and LSIL, they agreed as to the main possible choices that merited consideration: immediate colposcopy, cytologic follow-up and triage using HPV DNA testing.

Advances in HPV DNA testing have produced the first truly accurate and reproducible HPV assay systems.^{33,35,36} Accurate HPV DNA testing could be helpful in the management of ASCUS/LSIL in two ways. First, the type of HPV is strongly associated with the severity of squamous intraepithelial lesions and predicts the natural history of low grade lesions.^{4,6,11,15,18,20,23-25,29,44} Second, the presence or absence of cancer-associated types of HPV can help predict the accuracy of the original cytologic diagnosis of equivocal and low grade lesions in that HPV-negative patients are more likely to have false positive cytologic diagnoses.^{5,40}

With a mandate from a community of clinicians and researchers, NCI began the formal planning for ALTS in 1994.

Materials and Methods

Trial Objectives

ALTS was designed to evaluate three strategies for the triage of mild cervical cytologic abnormalities. The main end point of the study is the timely detec-

tion of all cases of CIN 3 or carcinoma. The strategy of immediate colposcopy is the reference standard of sensitivity. The specific objectives related to the other strategies are:

- To determine the effectiveness of cytologic follow-up of ASCUS and LSIL at six-month intervals.
- To assess whether the addition of one-time HPV testing to follow-up cytology can provide accurate and cost-effective triage for the Pap smear diagnoses of ASCUS and LSIL.

Eligibility Criteria

A woman was eligible to participate in ALTS if she:

- had a cytologic diagnosis of ASCUS or LSIL within six months of enrollment,
- was 18 years of age or older,
- had no prior hysterectomy,
- had no known history of ablative or excisional therapy to the cervix,
- was not pregnant, and,
- was able to provide informed consent and likely to participate for the full duration of the trial.

Summary of Trial Design

Potentially eligible women were referred from clinics affiliated with one of four clinical centers around the United States. Participants underwent an enrollment examination that included a pelvic examination, collection of cervical specimens for preparation of a liquid-based cytologic slide (ThinPrep™, Cytoc Corporation, Boxborough, Massachusetts, U.S.A.), and HPV DNA testing (Hybrid Capture II™, Digene Corporation, Gaithersburg, Maryland, U.S.A.) and the taking of cervical photographs (Cervigrams™, National Testing Laboratories, Fenton, Missouri, U.S.A.). Cervigrams™ are magnified visual images of the cervix taken after application of acetic acid and are reviewed off site by expert evaluators.^{9,38,41,43} Willing participants also provided a blood sample.

Patients were randomized to one of three arms. Women in the immediate colposcopy (IC) arm were referred for colposcopy, ideally within three weeks of enrollment. Women in the conservative management (CM) arm were referred to a colposcopist for any cytologic diagnosis of HSIL or carcinoma. Women randomized to HPV triage were sent for colposcopy if the enrollment HPV DNA test result was positive or missing (see below) or if the cytologic diagnosis at enrollment indicated HSIL or carcinoma.

Regardless of randomization arm and whether

colposcopy was done, all women are being followed every six months for two years. At each six-month follow-up visit, women undergo a pelvic examination, collection of cervical cells for the preparation of ThinPrep and masked HPV testing, and Cervicography™. Cytologic diagnoses of HSIL or carcinoma during follow-up leads to immediate referral for colposcopic examination. All women are scheduled for a colposcopic examination at the "exit" visit at 24 months.

To rule out carcinoma possibly missed by the clinical center procedures, a variety of safety nets are in place. National Testing Laboratories provides external review of the Cervigrams™. The rare P3 findings compatible with carcinoma are communicated immediately back to the clinical center, and the patient is referred for colposcopy if not already referred at that time for other reasons. A colposcopy quality control (QC) group monitors clinical center colposcopy by reviewing computerized digital images (Denvu, Tuscon, Arizona, U.S.A.) for carcinoma. An expert group of pathologists reviews cytologic and histologic slides and alerts the clinical center in cases of possibly missed CIN 3 or carcinoma. The HPV QC group monitors the quality of HPV testing. The guiding principle is to maximize the safety of participants while minimizing interference in clinical center management.

Organization of the Trial

NCI. Two project officers from the NCI, the sponsoring agency, provide overall scientific direction and oversight of the trial. The trial is funded as a group of eight contracts. The NCI project officers work with investigators from the four clinical centers, the coordinating unit and the QC groups to develop and implement the study protocol.

Clinical Centers. The four clinical centers are the University of Alabama in Birmingham, the University of Oklahoma in Oklahoma City, Magee-Womens Hospital of the University of Pittsburgh Medical Center in Pittsburgh, and the University of Washington in Seattle. Each center is responsible for establishing the procedures necessary to implement the basic protocol at their institution as well as recruiting, enrolling, and following study participants according to the protocol. Each center is based within existing gynecology and oncology clinics and staffed by a principal investigator, co-principal investigator, study manager, data manager, and clinical and administrative staff. Existing cy-

tology and histopathology laboratories associated with each hospital are utilized for the processing and local interpretation of all cytology and histology materials.

QC Groups. Three QC groups were selected to fulfill functions of safety and quality assurance. The pathology QC group, centered at Johns Hopkins University, provides central, expert review of all referral and enrollment cytology slides, selected follow-up cytology slides and review of all histology slides for the ALTS trial. Slide review is specific to the specimen type. For all referral and enrollment cytology slides, the pathology QC review protocol included a second screening by a pathology QC cytotechnologist (leaving both sets of dots in place) and review by a QC pathologist blinded to the original diagnosis. Any case with a diagnosis of HSIL, by pathology QC or clinical center, automatically went to an open panel review composed of two of the four QC pathologists. The QC panel pairings were chosen at random from among the four pathologists in each review cycle. For all other cases, the original (referral or clinical center) diagnosis was compared to the first QC review diagnosis, and, if concordant, that served as the final diagnosis. In the event of disagreement between the original and first QC reviewer, the case was sent to a second QC reviewer, blinded to both previous diagnoses. The three diagnoses were then compared. Any diagnosis of HSIL, ASCUS rule out HSIL or unsatisfactory went to panel review. Otherwise, if two out of three diagnoses were concordant, this served as the final diagnosis. Nonconcordant cases also went to panel review. For all cases sent to the panel, this review constituted the final diagnosis.

All histology slides were examined by a pathology QC reviewer. In case of disagreement between the first QC reviewer and the original clinical center diagnosis, discrepant cases were referred directly for panel review along with all possible high grade cases.

Follow-up cytology slides from 1998 and 1999 underwent a modified review process. A specially configured screening instrument (TriPath, Elon, North Carolina, U.S.A.) adapted for ThinPrep specimens first scanned all the slides. The following percentages of slides were then selected for rescreening by the cytotechnologist and review by the pathologist: 100% of the highest scoring top quintile (i.e., the 20% most likely to harbor disease), a random 25% of those that were classified as not technically

adequate for processing by the machine, and 5% of the slides that passed the screening process as "less likely to contain disease." Starting in 2000, 100% of follow-up slides were again reviewed by the pathology QC group.

The HPV QC group, headquartered at the University of New Mexico, is responsible for maximizing the accuracy and reliability of HPV testing throughout the trial. Prior to the start of enrollment, the HPV QC group evaluated a number of HPV tests and made recommendations as to the optimal test for ALTS. They established the control reagents and procedures for HPV testing throughout the trial. The group works to ensure the optimal application of the testing technique and performs QC retesting on a quarterly basis to evaluate the performance of the laboratories. The HC II test contains its own negative and positive controls. However, the HPV QC group introduced additional controls and procedures. To assess the accuracy of the test, the group developed "mock" replicate specimens and low-risk HPV controls that were assayed in each batch. The mock specimens used during enrollment consisted of HPV DNA of various types and concentrations mixed into a cellular matrix to simulate a cervical specimen. The expected values of the mock specimens were known only to the HPV QC group. The low-risk controls consisted of two concentrations of HPV type 53, aimed at the assessment of assay cross-reactivity. Mock specimens are not being used during follow-up due to the accurate and reliable performance of the HC II assay, as validated during enrollment and by ongoing retesting of a percentage of specimens.

The colposcopy QC group, based at the Medical College of Georgia, is responsible for monitoring the colposcopic assessments at the clinical centers. Prior to the beginning of the trial, this group conducted specific educational training, including the use of the Denvu 2 digital imaging system. They evaluated each participating colposcopist's skills by direct observation at the clinical centers and used the Colposcopic Recognition Award of the American Society for Colposcopy and Cervical Pathology as a colposcopy proficiency test. They also conducted focused reeducation as needed to ensure the proficiency of all trial colposcopists.

Digital images of the cervix taken during colposcopy are forwarded directly to one of three reviewers, who records a colposcopic impression and indicates the optimal site of a cervical biopsy if one is indicated. A second QC monitor reviews the dig-

ital images in the same masked fashion. Images are sent to a third reviewer for masked adjudication in the case of disagreement between the first two reviewers. Again, for the purposes of safety, any colposcopic impression consistent with carcinoma rendered by the QC team is immediately communicated back to the clinical center for appropriate follow-up of the trial participant. Other data are recorded for research purposes. Ongoing quality assurance is provided by means of routine comparisons between clinical center and QC group evaluations, evaluation of the quality of the images, on-site observations of colposcopic examinations performed by the clinical center colposcopists and educational activities as needed.

Coordinating Unit. To provide coordination of this complex trial, a coordinating unit (CU) was selected and established at Westat, Rockville, Maryland, U.S.A. Throughout the trial the CU has developed standardized manuals of procedures and data management systems and provided training to the clinical center staff on trial procedures. It has monitored trial activities, served as liaison between NCI and the other groups, and served as a repository for all trial data.

Steering Committee. A steering committee, consisting of principal investigators and key staff from NCI, the clinical centers, the CU and the QC groups, provides continued input into trial activities. Initially convening by telephone weekly and in person twice a year in Bethesda, Maryland, the Committee provided input as to the organization of the trial and the development of the research protocols. The steering committee continues to oversee issues of compliance with the clinical protocols, publications and other key trial issues.

Data and Safety Monitoring Committee. The data and safety monitoring committee (DSMC), an independent external panel, was appointed by the director of the Division of Cancer Prevention, NCI, to periodically review the trial data and to oversee issues of safety and proper conduct of the trial. The DSMC is composed of experts in the fields of gynecology, pathology, medical ethics, epidemiology and statistics, and patient advocacy.

Industry Collaborations. Industry collaborators include the following: Digene Corporation developed and supports the Hybrid Capture II HPV DNA test

Cytec developed and supports the ThinPrep cytologic technique, National Testing Laboratories provides training for Cervicography™ and evaluates the Cervigrams™, DenVu supports the Denvu digital colposcopy equipment and software, and Tri-Path supported the computer-assisted cytology system that was used to review follow-up ThinPreps collected during follow-up until the year 2000.

The Acknowledgments includes a list of key personnel from participating institutions.

Specific Methods

ALTS methods are compiled in a detailed procedures manual, which is updated when occasional

refinements of the protocol are necessary. For example, when an unanticipated situation arises that could occur again, an update is distributed. Otherwise, the protocol has remained fixed since the beginning of the trial, and the few violations to date are described in a log maintained by the CU. Some of the key elements of the protocol are described below.

Recruitment and Enrollment Procedures. Each clinical center had an established referral base consisting of gynecology, general practice and family planning clinics in its immediate geographical location from which potentially eligible women were identi-

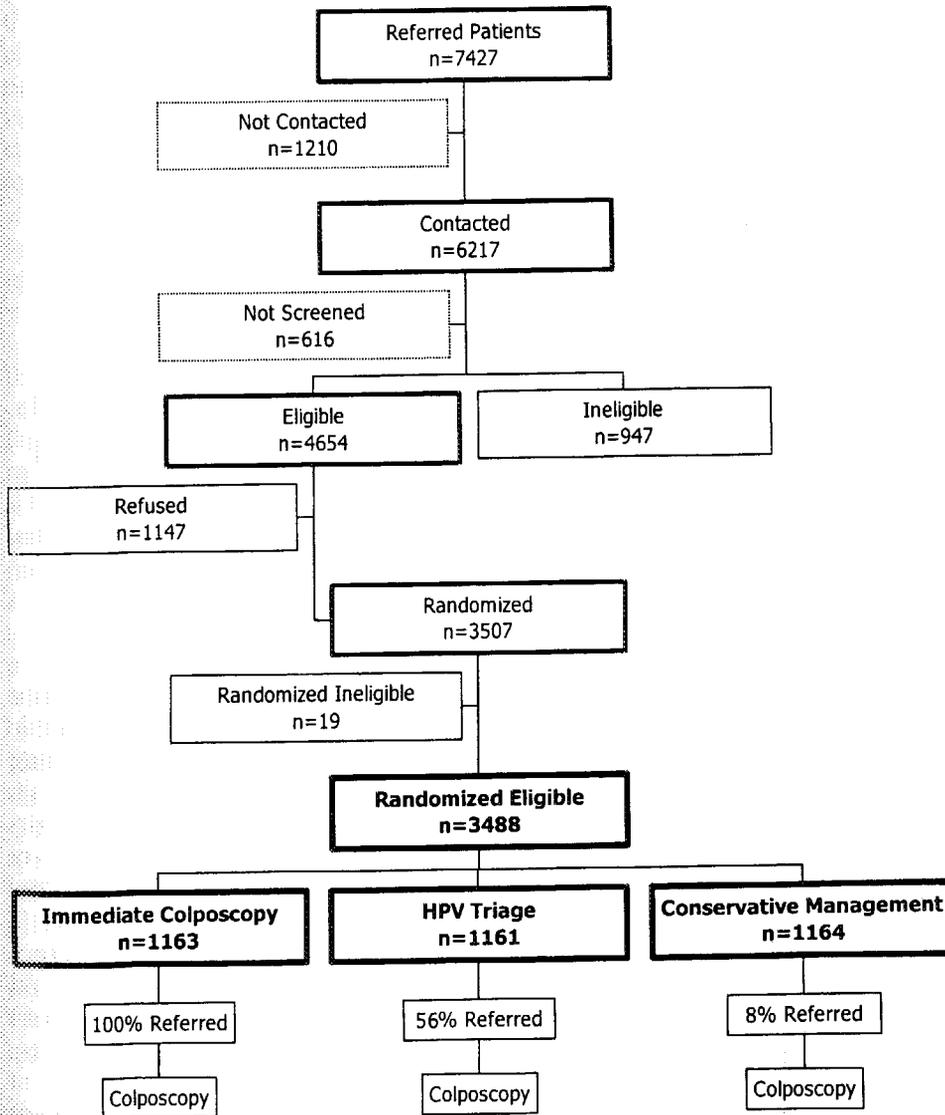


Figure 1 Ascertainment of eligibility, informed consent, randomization and triage to colposcopy at enrollment among women referred with a cytologic diagnosis of ASCUS.

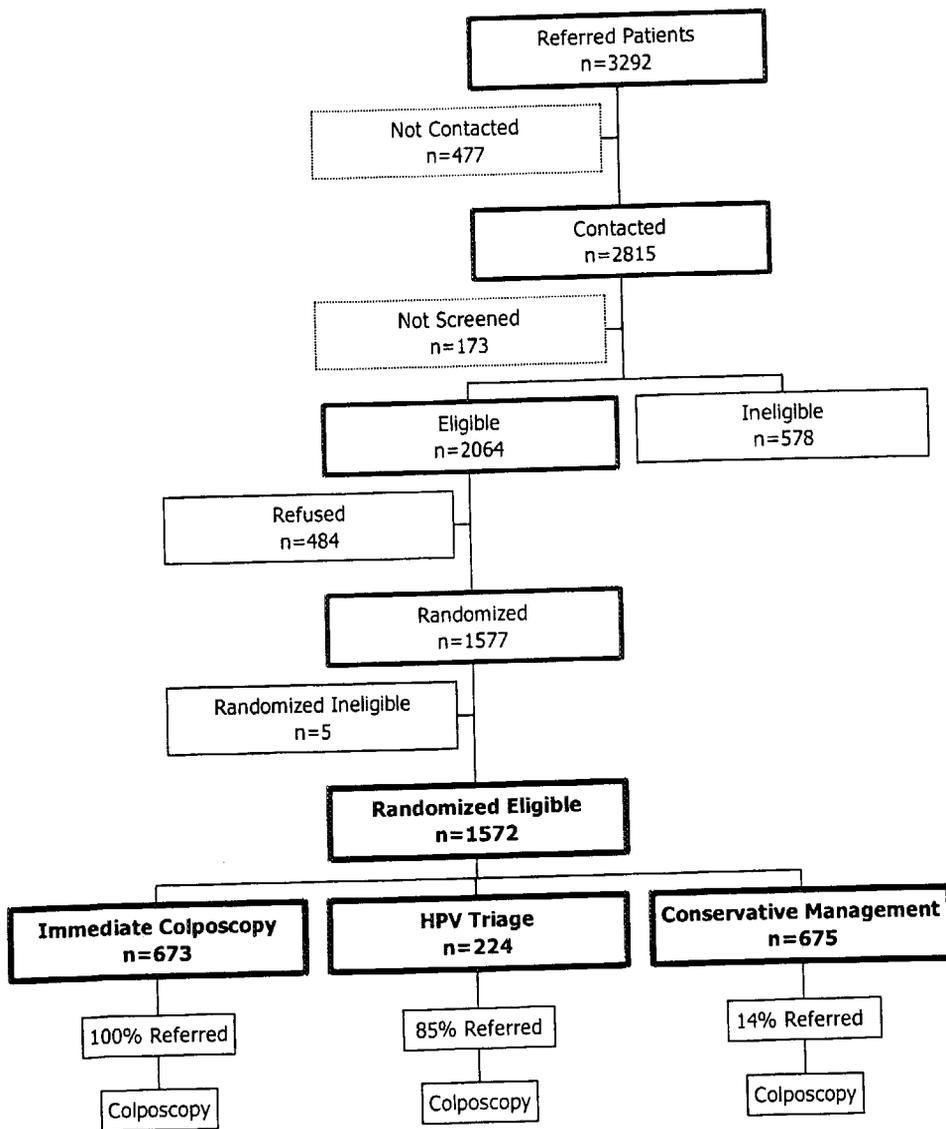


Figure 2 Ascertainment of eligibility, informed consent, randomization and triage to colposcopy at enrollment among women referred with a cytologic diagnosis of LSIL.

fied. From October 1996 to December 1998, each center either (1) arranged to review all Pap smear reports from women seen at referral clinics in order to identify ASCUS and LSIL cases, or (2) agreed to the referral clinics' prescreening the Pap smear reports for eligible ASCUS and LSIL cases. Women ultimately identified by the clinical center as potentially eligible (Figures 1 and 2) were first sent a letter and brochure describing the study and then telephoned by a recruiter and screened for eligibility using a standard set of questions. In order to compare characteristics of those who did not participate with those who agreed to participate, all potential participants whom we succeeded in contacting

were also asked a few demographic questions prior to being invited to participate in ALTS. Eligible women who expressed an interest in participating were given an appointment for an enrollment visit. Those who were found ineligible or refused participation were instructed on the follow-up necessary for abnormal Pap smears. Reasons for refusal were documented at all centers.

After their arrival at the clinic, women were provided with detailed information about ALTS and were asked to read and sign an informed consent form previously approved by the institutional review boards from participating institutions. Upon signing this document, the new ALTS participant

was given a study identification number and guided through the enrollment procedures. Very few instances of ineligibility or refusal occurred among women who came to the enrollment visits.

Questionnaire. Each ALTS participant was administered a standardized, precoded questionnaire to capture demographic, gynecologic, sexual, reproductive, medical and other information on health habits, such as intake of alcohol and smoking. Interviews were conducted in a private setting by a study staff member (often a nurse) trained to administer the study questionnaire. The average length of this interview was 11 minutes.

Pelvic Examination and Collection of Specimens. A nurse-colposcopist conducted the pelvic examination. The examination included, first, the inspection of the outer genitalia for the presence of gross abnormalities. Following insertion of a speculum, the vagina and cervix were inspected. If the vaginal discharge appeared abnormal, swabs were obtained for saline and KOH microscopic examinations, whiff tests, and other microbiologic culture and testing, as appropriate. Collection of the study specimens was occasionally postponed in cases of severe cervicitis or heavy menstrual flow.

Collection of the study specimens proceeded in the following order. First, a cervical cell sample was collected by inserting the long, central bristles of a Papette™ broom (Wallach Surgicals, Orange, Connecticut, U.S.A.) into the cervical os and rotating it 360° five times in one direction with sufficient pressure to bend the outer bristles against the ectocervix. Cells remaining on the Papette™ broom were collected in a PreservCyt™ vial (Cytyc) containing 20 mL of a methanol-based fixative by pressing the bristles against the bottom of the container and simultaneously twisting the handle. The broom was then discarded and the PreservCyt™ vial closed securely. In the case of a stenotic os, a second sample was obtained with a cervical brush and also rinsed into the same PreservCyt™ vial. A second sample of cervical cells was obtained for HPV DNA testing using a Dacron™ swab and placed in a vial containing specimen transport medium (STM, Digene). Following collection of the cervical cell specimens, a 5% solution of acetic acid was applied twice to the cervix, and two Cervigrams™ were taken.

Randomization. While the clinician completed the

examination and collection procedures, another ALTS staff person placed a telephone call to the randomization desk located at the CU. The participant was randomized to one of the three study arms using a computer system that required duplicate entry for verification of the assignment.

Women randomized to the IC arm proceeded immediately to colposcopy or were given an appointment to return for the procedure within three weeks if unable to stay the same day. Women randomized to the HPV arm were called back for a colposcopy visit only in the case of a positive or missing (see below) HPV test result at enrollment or a cytologic diagnosis of HSIL (or carcinoma). In the CM arm, only women with a cytologic diagnosis of HSIL were referred for colposcopy.

A missing HPV test result was most commonly due to insufficient (less than 4 mL) residual sample in the PreservCyt™ vial to perform the assay. For purposes of the trial, it was considered to be an impractical triage strategy to recall women for repeat collection for the HPV test alone. *A priori*, missing HPV test values were considered positive, biasing the triage result slightly toward higher sensitivity and lower specificity in order to reduce return visits. However, current standard management calls for repeating after inadequate cytologic specimens. Therefore, women with inadequate enrollment cytology results were recalled for repeat specimen collection (unless they were already triaged to colposcopy in the IC arm or in the HPV arm on the basis of a positive HPV test).

At the end of the enrollment visit, all patients, regardless of their randomization assignment, were asked to provide a 10-mL sample of blood for HPV immunology and other investigational assays. Samples were collected from the arm in a standardized fashion into heparinized tubes and shipped overnight at room temperature to the NCI's laboratory in Frederick, Maryland. Last, a request was made to the referral laboratories for the Pap smear slide that brought the participant into the trial for pathology QC review.

Processing and Interpretation of Enrollment Specimens. PreservCyt vials were taken daily to the clinical centers' cytology laboratories for the preparation of a liquid-based cytology slide (Cytyc ThinPrep™) according to the manufacturer's standard protocol. The ThinPrep processor agitates the 20-mL sample and draws cells onto a membrane filter by suction. When the filter has collected suffi-

cient cells to produce a slide, the suction is released, and the cells are transferred by positive pressure to a 20-mm, circular area on a glass slide and fixed. The prepared ThinPrep slides are stained according to routine practice, and a coverslip is placed. All ThinPrep slides are then screened by a cytotechnologist and evaluated by a cytopathologist according to routine practice. For ALTS, cytologic interpretation was conducted using the Bethesda System. Cytologic diagnoses were recorded on a standardized data collection instrument designed for ALTS. Following the clinical center interpretation, all enrollment ThinPrep slides were sent to the pathology QC group for further evaluation.

Following preparation of the ThinPrep, the remaining cervical sample in the PreservCyt vial was forwarded to the HPV laboratory for HPV testing.^{39,40,45} Four milliliters of sample was required for the assay. HPV testing was conducted in batches, every two weeks on average, using the Hybrid Capture II™ HPV DNA assay. PreservCyt™ specimens were aliquotted and denatured either the day of, or the day immediately preceding, the scheduled Hybrid Capture II™ assay. Each PreservCyt™ (Cytoc) vial was vigorously shaken by hand and immediately aliquotted to prevent settling of cellular material. A 4-mL aliquot of each specimen was transferred into a labeled, 10-mL conical tube (Sarstedt, Inc., Newton, North Carolina, U.S.A.) using a 5-mL serologic pipette. A 0.4-mL aliquot of Sample Conversion Buffer™ (Digene) was added to each specimen aliquot. The specimens, in batches of 20 or less, were vortexed and centrifuged in a swinging bucket rotor set to achieve $2,900 \pm 150$ g for exactly 15 minutes. The supernatant was decanted immediately following centrifugation to prevent diffusion of the cellular pellet. Residual supernatant was removed by blotting the inverted tube on an absorbant towel. A 150- μ L aliquot of two parts STM and one part sample denaturant (Digene) was added to each cellular pellet. The tubes were vortexed until the cellular material was fully resuspended. The samples were denatured in a 65°C water bath for 15 minutes, followed by additional vortexing, and returned to the water bath for 30 minutes. The denatured specimens were either immediately tested in the Hybrid Capture II™ assay or frozen at -20°C and assayed the following day.

Hybrid Capture II™ is a sandwich capture molecular hybridization assay that utilizes chemiluminescent detection: light is emitted in proportion to

the amount of target DNA in the specimen. The Hybrid Capture II™ assay is configured to detect, in a single assay, one or more of the following HPV types associated with a high risk of cervical carcinoma: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. A test is considered positive for the presence of HPV DNA if the relative light units (RLU) measurement (i.e., the measure of light emitted) is greater than or equal to the positive control, equal to 1 pg/mL of HPV 16 plasmid insert DNA per test reaction. An RLU measurement <1.0 pg/mL indicates either the absence of the specific HPV DNA types included in the cocktail or HPV DNA levels of high-risk types below the threshold of detection. The analytic sensitivity of the HPV Hybrid Capture II™ test is approximately 5,000 genome equivalents per assay well for one or more of the 13 HPV types included in the high-risk cocktail. The Hybrid Capture II™ assay is known to detect, albeit with decreased sensitivity, some low-risk HPV types (particularly types 53 and 66) that are genetically related to the high-risk types.³³

The second sample of cervical cells in STM was stored at the clinical centers and shipped on a monthly basis to the NCI biorepository, located in Frederick, Maryland, for subsequent HPV typing by polymerase chain reaction (PCR)-based methods.^{12,14,27,35} HPV typing is being used for selected investigational analyses, not for clinical management.

Enrollment Colposcopic Examination. The enrollment colposcopic examination was performed by a gynecologist or nurse-colposcopist. The standard protocol included conventional visual assessment, application of 5% acetic acid, identification of the squamocolumnar junction and transformation zone, recognition of suspected neoplastic lesions and notation of their linear extent, size and severity. The overall colposcopic impression was defined as normal, atypical metaplasia, cervicitis, low grade disease (CIN 1, HPV), high grade disease (CIN 2, CIN 3/CIS) or carcinoma (squamous or adenocarcinoma). The colposcopist used the DenVu computer-assisted digital imaging system to capture images of the cervix at low (10 \times) and high power (16 \times) magnification. Colposcopically directed cervical biopsies were obtained of any lesion suspicious for SIL, taken in order from worst to least severity and recorded on the digital image. Endocervical curettage was performed according to the clinician's judgment in cases where the transformation zone or

proximal extent of a cervical lesion was not adequately visualized.

Handling of Colposcopic Materials. Biopsies taken during the colposcopic procedure were placed in separate, labeled vials containing 10% buffered formalin and taken directly to the histopathology laboratory for routine processing. Histologic interpretation was conducted using a modification of the Bethesda System. Findings were recorded on a standardized data collection instrument designed for ALTS. Following the interpretation by the local histopathology laboratory, all histology slides were sent to the pathology QC group for further evaluation.

Digital Images. Digital images of the cervix were sent automatically by modem from each clinical center to members of the colposcopy QC group during off-peak hours each night.

Management of Participants Undergoing Colposcopy and Treatment of Histologically Confirmed Lesions. All participants who underwent colposcopy at or following enrollment were managed the same way, regardless of the path by which they arrived at colposcopy. Women with histologically confirmed HSIL (CIN 2 and 3) were treated by LEEP. This excisional treatment option was selected over alternatives, such as cryosurgery, in order to ensure a definitive pathologic specimen for final diagnosis. Women with histologically confirmed LSIL were not treated and are being followed in a prospective, cohort study.

Follow-up Visits

Regardless of randomization arm, participants are scheduled to return to the clinic every six months for two years for routine follow-up. At the 6-month, 12-month, 18-month and 24-month (exit) visits, trial participants are administered a follow-up questionnaire to capture new medical and risk factor information since the previous visit. Additionally, at the 12-month visit, women complete a self-administered quality-of-life instrument.

At all follow-up visits participants undergo a pelvic examination similar to the enrollment examination, have cervical cells collected for the preparation of ThinPrep™ and masked HPV testing, and have two Cervigrams™ taken. At the six-month visit, the first 1,500 women also had a conventional Pap smear prepared for purposes of comparison

with the ThinPrep™, using a “split-sample” design in which the conventional smear was prepared first and then the collection instrument was rinsed into a PreservCyt™ vial. Given the equivalent sensitivity of the two methods for the detection of HSIL (data analysis not shown), all subsequent cytologic slides were prepared as ThinPrep™ slides. Throughout follow-up, only the ThinPrep™ cytology results are being used for referral to colposcopy. HPV test results are masked during follow-up, even in the HPV arm.

For safety, all trial participants, regardless of randomization arm, are receiving a colposcopic examination and, if indicated, colposcopically directed biopsy at the exit visit. At this visit alone, all the available clinical center and QC cytologic and histologic data, as well as the HPV results, are unmasked to inform the colposcopist of the subject's full history. Moreover, the last available Cervigram™ (in the form of a photograph) is provided to the clinician performing the exit visit examination. Women found to have HSIL (CIN 2/3) on cytology or histology are being treated with LEEP. Those with a histologic diagnosis of CIN 1 are also treated with LEEP if they have persistent cytologic or histologic LSIL/CIN 1 or persistent cytologic ASCUS with a positive HPV Hybrid Capture II™ test unless the clinician and/or patient declines. The decision to treat women with persistent mild lesions, especially with oncogenic HPV infections, was made based on the increased risk of high grade lesions, including CIN 3, shown by recent natural history studies.^{15,29}

Cost Utility Study

The quality of life and cost-effectiveness studies, headquartered at Dartmouth Medical School, will ascertain the impact on women's lives of the three triage strategies in ALTS. The incremental costs of these management strategies will be compared.

Data Processing

Initial Data Handling. All ALTS data collected at the clinical centers are recorded by clinical center staff on standardized data collection forms designed especially for ALTS. All questionnaires are sent directly from the centers to the CU for review, coding and double data entry. Data retrieval questions are sent to the clinical centers only on variables considered to be vital to the analyses.

Nonquestionnaire data are keyed and edited at each of the clinical centers using a data manage-

ment system developed by the CU for ALTS. Collected data are transmitted on a monthly basis to the CU, where additional edit checks are performed. Data are also received and checked similarly on a routine basis from the QC groups and National Testing Laboratories, each using a different data management approach. (A separate database system was created for pathology QC, whereas the colposcopy QC group created and maintains its own database.)

Anticipated Data Analysis. ALTS data will be published in two waves, corresponding to the end of enrollment and the end of follow-up. The anticipated publications are quite varied. The major analyses will describe the sensitivity and specificity of the various triage strategies, with reliance on primary randomization. However, multiple other papers will analyze aspects of ALTS apart from randomization. Examples based on enrollment data include estimating the risk of underlying CIN 3 in women with ASCUS or LSIL based on all available data, determining optimal cut-points for use of Hybrid Capture II™ DNA testing (1.0 pg/mL may not always be the best cut-point) and describing the subset of ASCUS that resembles HSIL.

Primarily, ALTS was designed to have excellent power to detect a small difference in the percentage of management failures (delayed detection of CIN 3+) in the HPV and CM arms as compared to the IC arm. CIN 3+ could be prevalent at enrollment or arise incidently during follow-up. We estimated *a priori* that approximately 10% of LSIL and 5% of ASCUS referral Pap smears would indicate underlying CIN 3+ during the trial, either at enrollment or during follow-up. We assumed that immediate colposcopic examination would be virtually 100% sensitive in detecting all CIN 3+ in that arm.

The HPV triage arm was closed for LSIL referrals in the first year of the trial because the great majority of women with LSIL tested positive (limiting the possible triage utility of HPV testing).¹ The final analyzed enrollment data set includes 3,488 women with ASCUS in three arms and 1,572 women with LSIL, mainly in two remaining arms. These numbers nearly matched the goals for ASCUS recruitment (1,200 per management arm) but fell short of the LSIL goals.

Stopping Rules. The data and safety monitoring committee reviews all salient trial data every six months to judge whether the trial is safe and worth

continuing. The most difficult safety issue is how to analyze the sensitivity of cytology with regard to the timely detection of CIN 3+. In the United States, cytologic screening is designed as a repeated program, not a one-time event. Thus, intrinsic to the CM arm is the concept that most cases of ASCUS and LSIL will regress and those cases with prevalent or evolving CIN 3+ will be detected by repeat cytology. As a corollary, immediate colposcopy leading to biopsy and treatment, although the reference standard of sensitivity, would expose most women (millions in absolute numbers) to the risk of excessive treatment. The data and safety monitoring committee exercises its judgment in balancing the needs of the trial with the safety of participants.

Results

Recruitment

The structure of recruitment, randomization, enrollment and follow-up is illustrated in Figures 1 and 2.

Between January 1997 and December 1998, a total of 7,427 women with ASCUS and 3,292 women with LSIL were referred to the four clinical centers. Because the centers differed in whether potential subjects were prescreened for eligibility by the referral clinics, the number of referrals cannot be strictly compared across centers. Moreover, the community study population cannot be strictly estimated. The ratio of ASCUS:LSIL varied from approximately 1:1 to 3.5:1 by center. The enrollment rates among referred women did not differ by referral diagnosis. Participants tended to be slightly older and marginally better educated and were slightly more likely to be white or Asian as compared to nonparticipants.

As shown in Figures 1 and 2, approximately two-thirds of the referred women were successfully contacted and found to be eligible. Of the eligible potential participants, about 75% agreed to participate and came to the clinic. Virtually all women who came to the clinic were ultimately randomized and enrolled.

The characteristics of enrolled participants are shown in Tables I-III. The final enrollment data set discussed here includes 5,060 women because 24 randomized women had no enrollment examination and/or interview. Data for ASCUS and LSIL referrals are given separately in the tables, but the trial arms and clinical centers are combined. Although the participants from the different clinical

Table I Demographic Characteristics of Enrolled Participants, by Referral Diagnosis

Characteristic	ASCUS		LSIL	
	n	%	n	%
Age (yr) (n = 5060)	3,488	100	1,572	100
18–20	667	19.1	478	30.4
21–23	683	19.6	404	25.7
24–27	693	19.9	326	20.7
28–34	661	19.0	229	14.6
35 +	784	22.5	135	8.6
Race (n = 5024)	3,463	100	1,561	100
White	2,201	63.6	990	63.4
Black	1,080	31.2	475	30.4
Native American/ Alaskan native	65	1.9	43	2.8
Asian/Pacific Islander	117	3.4	53	3.4
Hispanic (n = 5052)	3,480	100	1,572	100
Yes	159	4.6	71	4.5
No	3,321	95.4	1,501	95.5
Highest Level of Education (n = 5057)	3,487	100	1,570	100
Elementary	519	14.9	296	18.8
High school/GED	1,057	30.3	495	31.5
Vocational/some college	1,316	37.7	588	37.5
Completed college	432	12.4	138	8.8
Some graduate work	163	4.7	53	3.4

centers differ in most respects (e.g., mean age, race and ethnicity, frequency of various behaviors, prevalence of cervical neoplasia), this heterogeneity does not modify our conclusions regarding the risk factors for CIN 3+. For example, the very high prevalence of HPV DNA found in prevalent cases of LSIL¹ was evident uniformly at all centers. Most important, randomization was effective at each clinical center. We found no significant differences between the participants in the three trial arms with regard to any of the variables shown in the tables.

Demographic Characteristics of Enrolled Participants

As expected, the ALTS population was generally young, with a mean age of 27 years (median, 25; range, 18–81). Women referred for LSIL were younger (mean age, 25) than those referred for ASCUS (mean age, 29; $P < .001$ for test of independent means). Race and ethnicity did not differ by referral diagnosis. However, women referred for LSIL tended to report fewer years of schooling than those referred for ASCUS. This initially significant difference was explained by the younger age of the women referred for LSIL. (We adjusted for the con-

founding effects of age by using multivariable logistic regression, with age treated as a categorical variable.)

Behavioral Characteristics of Enrolled Participants

Table II shows selected behavioral characteristics of the ALTS population at enrollment by referral diagnosis. Two conventionally strong risk factors for cervical carcinoma and SIL did not differ as might be predicted between the referral groups. Women referred for SIL tended to initiate sexual intercourse about the same time (average age, 16–17) and to have no more sexual partners than women referred for ASCUS once current age was taken into account.

Overall, the overwhelming majority of participants (98%) reported some use of hormonal contraceptives. Seventy-five percent reported using hormonal contraceptives in the two years prior to

Table II Behavioral Characteristics of Enrolled Participants, by Referral Diagnosis

Characteristic	ASCUS		LSIL	
	n	%	n	%
Age at first sexual intercourse (n = 5038)	3,471	100	1,567	100
≤ 14	596	17.2	291	18.6
15	510	14.7	251	16.0
16–17	1,265	36.4	647	41.3
18+	1,100	31.7	378	24.1
Lifetime no. of sex partners (n = 4990)	3,442	100	1,554	100
1–2	625	18.2	236	15.2
3–4	786	22.9	382	24.6
5–6	694	20.2	323	20.8
7–12	712	20.7	359	23.1
13+	620	18.0	253	16.3
Use of contraceptives in past 2 yr (n = 5057)	3,487	100	1,570	100
Hormonal	2,459	70.5	1,249	79.6
Physical barriers	2,353	67.5	1,203	76.6
Other	1,591	45.6	738	47.0
No. of live births (n = 5057)	3,486	100	1,571	100
0	1,406	40.3	700	44.6
1	856	24.6	427	27.2
2+	1,224	35.1	444	28.3
Smoking status (n = 5059)	3,488	100	1,571	100
Never	1,900	54.5	783	49.8
Former	464	13.3	152	9.7
Current	1,124	32.2	636	40.5
No. of Pap smears in previous 5 yr (n = 5038)	3,474	100	1,564	100
0–3	1,135	32.7	622	39.8
4–5	1,672	48.1	641	41.0
6+	667	19.2	301	19.2

Table III Clinical Center Cytology and HPV Hybrid Capture II™ Result at Enrollment, by Referral Diagnosis

Parameter	ASCUS		LSIL	
	n	%	n	%
Enrollment cytology (n = 5060)	3,488	100	1,572	100
Unsatisfactory	18	0.5	6	0.4
Negative	816	23.4	158	10.1
Reactive cellular changes	644	18.5	136	8.7
ASCUS	1,135	31.6	364	23.2
NOS	279	8.0	79	5.0
Favor reactive	375	10.8	91	5.8
Favor LSIL	376	10.8	166	10.6
Metaplastic	105	3.0	28	1.8
LSIL	630	18.1	709	45.1
HSIL	245	7.0	198	12.6
NOS	43	1.2	21	1.3
CIN 2	163	4.7	156	9.9
CIN 3	39	1.1	22	1.4
Hybrid Capture II™ test result (n = 5060)	3,488	100	1,572	100
Missing	164	4.7	79	5.0
Negative	1,558	44.7	237	15.1
Positive	1,766	50.6	1,256	79.9

enrollment. This differed significantly by referral diagnosis, with 80% of those referred with LSIL as compared with 71% of those referred with ASCUS reporting use ($P < .001$ by standard χ^2). Use of barrier methods was also significantly higher in those referred with LSIL (77% versus 67%, $P < .001$).

Over half of ALTS participants reported at least one live birth. Women referred with LSIL reported fewer live births as compared to those referred with ASCUS; that difference was explained by the age difference between the groups.

Cigarette smoking (ever) was reported by about half the participants, although most of the smokers were no longer smoking. Women with LSIL were marginally more likely than women with ASCUS to be current smokers, even when age was taken into account.

Women referred for LSIL had slightly fewer Pap smears in the previous five years than those referred for ASCUS, even adjusting for age.

There were no striking differences between the two referral groups in past histories of other sexually transmitted diseases. Overall, 21% of women reported a history of *Chlamydia trachomatis*, 13% of women reported having had vulvar warts, 13% reported a diagnosis of *Trichomonas vaginalis*, 8% reported *Neisseria gonorrhoeae*, 6% gave a history of

genital herpes simplex virus, and 1% reported syphilis.

Clinical Results at Enrollment

Women were enrolled in ALTS within an average of two months from the date of the ASCUS or LSIL diagnosis that initiated the referral to ALTS (median, 52 days, range, 8–184 days). The average referral lag time did not vary significantly by diagnosis or study arm.

Based on the enrollment thin-layer cytology as prepared and interpreted by the clinical center, 42% of women referred for ASCUS were diagnosed at enrollment as having normal or benign cytology, 32% were diagnosed with ASCUS again, and 25% showed a worse cytologic diagnosis (Table III). Women referred with a diagnosis of LSIL had more severe enrollment cytologic diagnoses than those referred for ASCUS. Specifically, among women referred for LSIL, only 19% had a negative or reactive cytologic diagnosis at enrollment, 23% were diagnosed as ASCUS, 45% had repeat LSIL, and 13% were diagnosed with HSIL ($P < .001$).

The enrollment Hybrid Capture II™ results are shown in Table III, stratified by referral diagnosis. HPV positivity in women with LSIL referral diagnoses was so high that it precluded efficient use of HPV testing as a triage tool for this diagnosis.¹ This led to early closure of the HPV arm of the trial for women with LSIL; i.e., women were randomized subsequent to the closure into either the CM or IC arm only (as reflected in Figure 2).

Colposcopic referral rates varied substantially, as expected, by arm of the study and referral diagnosis (Figures 1 and 2). The percentage of women referred for colposcopy by the study protocol who actually attended the ALTS colposcopy clinics was extremely high (>90%) in all arms (Figures 1 and 2).

The prevalence of CIN 3+ found in ALTS at enrollment generally matched the *a priori* expectations. Details will be published in the coming months as part of the discussion of enrollment cytology and HPV test sensitivity (Solomon, in preparation).

With approximately one-third of women having completed the ALTS protocol, follow-up rates have averaged 65–80% at the various centers for each of the 6-, 12-, 18- and 24-month (exit) visits.

Discussion

ALTS is a demanding, collective effort that will prove its worth only if the results are clear and valu-

able to both clinicians and researchers. To date, the large number of enrolled women, successful randomization and high rates of follow-up are very encouraging. The first publications from the enrollment phase of the study will emerge within the next year. The main analyses of the completed follow-up data will be published by 2002.

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