ASSESSMENT OF GENITAL HUMAN PAPILLOMAVIRUS (HPV) INFECTIONS AS A HEALTH PROBLEM IN RUSSIA, BELARUS AND LATVIA. MEASURES NECESSARY FOR THEIR EARLY DETECTION, TREATMENT AND CONTROL OF CERVICAL CANCER AND ITS PRECURSOR (CIN) LESIONS.

(Contract # ERB IC15-CT98-0321)

Stina Syrjänen1, DDS, PhD; Irena Shabalova2,3, MD, PhD, MIAC; Nicolay Petrovichev2, MD, ScD; Vladimir Kozachenko2, MD, ScD; Tatjana Zakharova2, MD, PhD; Julia Pajanidi4, MD, PhD; Jurij Podistov2, MD, PhD; Galina Chemeris2, MD, PhD; Larisa Sozaeva3, MD, PhD; Elena Lipova3, MD, PhD; Irena Tsidaeva4, MD; Olga Ivanchenko4, MD; Alla Pshepurko4, MD; Sergej Zakharenko5, MD; Raisa Nerovjna6, MD; Ludmila Klijukina7, MD, ScD; Oksana Erokhina7, MD; Marina Branovskaja8, MD, PhD; Maritta Nikitina8, MD, PhD; Valerija Grunberga9, MD; Alexandr Grunberg9, MD; Anna Juschenko9, MD; Marcella Cintorino10, MD, PhD; Rosa Santopietro10, MD, PhD; Piero Tosi10, MD, PhD; and Kari Syrjänen10,11 MD, PhD, FIAC

1Department of Oral Pathology, Institute of Dentistry, and MediCity Research Laboratory, University of Turku, Turku, Finland; 2N.I. Blokhin Cancer Research Centre of Russian Academy of Medical Sciences (RAMS), Moscow, Russia; 3Russian Academy of Post-Graduate Medical Education, Moscow, Russia; 4Novgorod Clinical Regional Hospital, Centralised Cytology Laboratory, Novgorod, Russia; 5Novgorod Municipal Dermatovenereological Dispensary, Department of Gynaecology, Novgorod, Russia; 6Novgorod Female Consultative Outpatient Hospital, Department of Gynaecology, Novgorod, Russia; 7Research Institute of Oncology and Medical Radiology, Republican Centre of Clinical Cytology, Minsk, Belarus; 8Minsk State Medical Institute, Department of Gynaecology and Obstetrics, Minsk, Belarus; 9Latvian Cancer Centre, Department of Gynaecology, & Laboratory of Cytology, Riga, Latvia 10Department of Pathology, University of Siena, Siena, Italy; 11Unit of Cytopathology, National Centre of Epidemiology, Surveillance and Promotion of Health, National Institute of Health (ISS), Rome, Italy.
ASSESSMENT OF GENITAL HUMAN PAPILLOMAVIRUS (HPV) INFECTIONS AS A HEALTH PROBLEM IN RUSSIA, BELARUS AND LATVIA. Measures Necessary for Their Early Detection, Treatment and Control of Cervical Cancer and Its Precursor (CIN) Lesions.

Background: To date, over 80 different human papillomavirus (HPV) types have been recognised and characterized, and a significant risk for the development of invasive cervical cancer has been ascribed to infections of the high-risk HPV types (IARC 1995). The natural history of cervical HPV infections seems to be identical with that of cervical precancer lesions (Syrjänen, 1997). An invasive cervical cancer invariably develops through these well defined precursor lesions (dysplasia, CIN), the early detection and eradication of which is a prerequisite for a successful prevention of cervical cancer. Although cervical cancer remains to be a major health problem worldwide (>500,000 new cases/year), it still is the only malignant human tumour which can be effectively prevented by medical intervention, as shown by the data e.g. from Finland, where an organized mass-screening programme has reduced the incidence of invasive cervical cancer to the lowest figures in the world, 2.7/10^5 (Syrjänen, 1997; Hakama, 1997). Because of the fact that organized mass-screening programmes are not realistic in most of the developing countries (including NIS/CCE), other measures should be searched for to alleviate the cancer burden in these areas, as recently recommended by the joint expert conference (WHO&EUROGIN)(Franco et al., 1996; 1997). Such alternative measures suggested include e.g. the screening of genital HPV infections by the available new molecular diagnostic tools (hybridization and PCR).

Objectives: In the present proposal, an approach is made 1) to assess the magnitude of genital HPV infections, CIN and cervical cancer as a health problem in the participating NIS/CCE countries, 2) to disclose the risk groups for this disease within these countries, 3) to elucidate the optimal (and the minimum) requirements for an appropriate detection of these risk groups by taking into account the local (and limited) resources, and finally 4) by combining the research and development component of this project, to create an effective health care system in the participating NIS/CCE centers, capable of independently diagnosing and treating genital HPV and CIN, with the decreased disease burden due to cervical cancer as the main long-term objective. A potential European dimension will be provided by the future implementation of such a system in other European countries with cervical cancer as a major health problem.

Study Design: This study is a classical population-based, cross-sectional (prevalence) study combined with a prospective cohort study. Thus, during the first year of this project, consecutive series of women attending the participating centers in the NIS/CCE are screened for cervical HPV infections and CIN, and the positive women (=prevalence data, geographic variation) will then comprise the cohort to be prospectively followed-up in these centers up to the end of this 3-year project (=disease prognosis). To assess the different levels of risk for HPV and CIN, the study is focused on two target populations of women in the NIS/CCE; 1) those attending the Gyn. Obst. clinics, and 2) those attending the STD clinics. The detection of the disease will be completed through two arms: 1) by PAP smears (ARM I), and 2) by PCR (ARM II). This enables the comparison of the efficacy, feasibility and cost-effectiveness of the new molecular diagnostic tools (PCR, ISH) with the traditional HPV/CIN detection methods (PAP smear, colposcopy, biopsy). PCR-negative women will constitute the Control Group for all HPV/CIN women, to be analysed for the epidemiological risk factors by questionnaires. Depending on the severity of the lesion on biopsy, the women are allocated to two mutually exclusive outcomes: 1) prospective follow-up, and 2) prompt treatment.

Partners and Coordination: This proposal is a joint research project, contributed by three centers in two EC countries (Finland, Italy), and 5 centers from three NIS/CCE countries (Russia, Belarus, Latvia), with the Coordination in Kuopio, Finland and Scientific Coordination in Moscow, Russia.

Significance: The main significance of the proposed cooperation will be in the fact the existing but clearly ineffective health care system in the NIS/CCE will be developed more efficient to cope with this major health problem. This can only be achieved by a joint research project with the expertise in the EC and utilizing their existing health care system as a reference.
THE ORIGINAL RESEARCH PLAN

B2. DETAILED DESCRIPTION OF THE PROPOSED ACTIVITY

1. PRESENT STATE OF KNOWLEDGE IN THE PROPOSED RESEARCH

Before the late 1970’s, the meager interest in epidemiology of Human papillomavirus (HPV) infections was focused on external genital warts (Condylomata acuminata), suspected to be a sexually transmitted disease (STD) since Antiquity (Oriel, 1971; 1981), and convincingly proved to be so in 1954 (Barrett et al., 1954). The demonstration of HPV particles in genital warts some 15 years later (Dunn et al., 1968; Almeida et al., 1969) confirmed the viral etiology of this common STD. The late 1970’s witnessed a major breakthrough by the light microscopic description of koilocytosis (the cytopathic effect of HPV, regularly present in exophytic condylomas) in the flat epithelial lesions as well, thus linking HPV with the precancer lesions of the uterine cervix and other genital mucosa (Koss et al., 1956; Meisels et al., 1976; Purola et al., 1977). Similar morphological evidence was soon reported also in invasive cervical carcinomas (Syrjänen, 1979), supporting the concept on a possible causal role of HPV in cervical carcinogenesis, as proposed a few years before (zur Hausen 1976).

Bursted by these key clinicopathological observations, an expansion of HPV research has followed since the early 1980’s, continuing to date and having significantly contributed to our current understanding of the etiology and pathogenesis of cervical squamous cell carcinogenesis. To date, almost 80 different HPV types have been recognised and characterized, and a significant risk for the development of invasive cancer has been ascribed to infections of the high-risk HPV types (Syrjänen et al., 1987; de Palo et al., 1988; von Krogh et al., 1989; Gross et al., 1990; 1997; Monsonego, 1990; 1995; Munoz et al., 1990; 1992; Crum et al., 1991; zur Hausen, 1994; IARC 1995; Mindel, 1995).

Apart from the molecular and clinical studies, significant new data to support the causal role of HPV in genital carcinogenesis have been obtained by epidemiological means, providing data on the prevalence (2-3%) and incidence (8%) of HPV infections, the risk factors for HPV infections and genital precancer lesions, as well as on the determinants of progression of these precancer lesions towards an invasive disease (Syrjänen et al., 1984; 1990a; Munoz et al., 1988; Syrjänen, 1989; zur Hausen, 1989; Beutner et al., 1991; Koutsky, 1991; Koutsky et al., 1988; 1989; Bosch et al., 1989; 1992; 1994; Kiviat et al., 1993; Fisher, 1994; Ferenczy, 1995; Schiffman, 1994; Schiffman et al., 1993; 1995; Palefsky et al., 1995; Vittorio et al., 1995; Burger et al., 1996; Duggan, 1996; Morris et al., 1996; zur Hausen 1996a; 1996b). Not unexpectedly, the risk factors for genital HPV infections are identical to those predisposing the women for an invasive cervical cancer, i.e., the characteristics mostly associated with the sexual behaviour, thus conferring a potential risk for the development of genital precancer lesions in both sexual partners (Barrasso et al., 1987; Campion et al., 1985; Hippeläinen et al., 1991; 1993; 1994; Syrjänen, 1995a).

Another key issue in understanding the epidemiology of HPV infections is the assessment of their natural history (Syrjänen, 1986; 1989; 1992; Syrjänen et al., 1985; 1988; 1990a). Evidence on the definite progressive potential of cervical HPV lesions has been obtained by prospective cohort studies (Hellberg et al., 1994; Raisi et al., 1994; Morrison, 1994; Downey et al., 1994; Kenemans, 1994; Terry et al., 1994). On the other hand, histologically documented regression has been established for a significant proportion of genital HPV infections in such studies (Syrjänen, 1990; 1996; Syrjänen et al., 1985; 1988; 1990a; Kataja et al., 1992; 1993). Thus, the natural history of cervical HPV infections seems to be identical with
that of the precancer lesions, as established by a large number of cohort studies during the past several decades (Östör, 1993; Syrjänen, 1997).

It has been established beyond doubt that an invasive cervical cancer develops through the well defined precursor lesions (dysplasia, CIN or SIL). It is equally clear that early detection and eradication of these cancer precursors is a prerequisite for an effective prevention of cervical cancer (Gross et al., 1990; 1997; Monsonego, 1990; 1995; Munoz et al., 1990; 1992; Crum et al., 1991; zur Hausen, 1994; IARC 1995). Although cervical cancer remains to be a major health problem worldwide (some 500,000 new cases/year), it still is the only malignant tumour which can be effectively prevented by medical intervention, as shown by the data from the Nordic countries, where organized mass-screening programmes have been implemented since 1960's. The most successful in this this respect has been the mass-screening programme in Finland, where the incidence of invasive cervical cancer has declined to one fourth since the early 1960's, currently representing the lowest figures in the world, 2.7/10^5 (Syrjänen, 1995b; 1997; Hakama, 1997). Few countries are that fortunate, however, and incidence figures as high as over 40/10^5 are continuously recorded in the high-risk countries (Munoz et al., 1990; 1992; IARC 1995, Franco et al., 1997). Because of the fact that organized mass-screening programmes are not realistic in most of these developing countries, other measures should be searched for to alleviate the cancer burden in these areas, as recently recommended by the joint expert conference (WHO&EUROGIN)(Franco et al., 1996; 1997). These measures include, downstaging, unaided visual inspection (UVI), colposcopy, cervicography, and screening of genital HPV infections by different molecular diagnostic tools.

In the present proposal, an approach is made 1) to assess the magnitude of genital HPV infections, CIN and cervical cancer as a health problem in the participating NIS countries, 2) to disclose the risk groups for this disease within these countries, 3) to elucidate the optimal (and the minimum) requirements for an appropriate detection of these risk groups by taking into account the local (and limited) resources, and finally 4) by combining the research and development component of this project, an effective health care system could be created to the participating NIS centers, capable of independently diagnosing and treating genital HPV and CIN, with the decreased disease burden due to cervical cancer as the main long-term objective in the future.

2.OBJECTIVES OF THE PROPOSED RESEARCH

2.1.Background and Reasoning

With the prevalence of 2-3% among the general population and with the annual incidence of 8% among young (22-year-old) sexually active women, cervical HPV infections currently represent the single most common STD worldwide (Syrjänen et al., 1990b; 1990c). The high-risk HPV type 16 has been recently proclaimed as definitely carcinogenic to humans by IARC (1995). Indeed, epidemiological evidence is overwhelming to confirm HPV infection as the most important risk factor for cervical cancer (IARC, 1995). Invasive cervical cancer remains to be the second most frequent female malignancy worldwide, and number one killer in the developing countries. Significant global geographic variation exists in the incidence of cervical cancer; low-, intermediate-, and high-risk areas being clearly recognized (Munoz et al., 1990; 1992; IARC, 1995; Franco et al., 1997).

Unlike all other human malignancies, however, cervical cancer is a potentially preventable disease. This can be theoretically achieved by two different approaches: 1) eradicating the
etiological agent (HPV), and 2) by organized early detection of cancer precursors. The first alternative is currently on the stage of experimentation, while vaccination trials are ongoing in limited areas (Munoz et al., 1990; 1992; IARC, 1995; Franco et al., 1997). The second approach has definitely proven its efficacy as organized mass-screening programmes successfully implemented in the Nordic countries (Sweden, Finland, Iceland). With such programmes, a significant reduction in the incidence and mortality has been achieved by using a simple and inexpensive test, the Papanicolaou cervical smear (Syrjänen, 1995b; Hakama, 1997; Franco et al., 1997). Unfortunately, the obstacles for the establishment of such programmes are overwhelming in most developing countries, where facilities for clinical cytology do not exist. For such regions, other measures have been recommended by a recent Joint Consensus Conference of WHO/EUROGIN in Geneva, including unaided visual inspection (UVI), cervicography, and testing for genital HPV infections by new molecular diagnostic tools (hybridization or PCR) (Franco et al., 1996; 1997).

In this respect, the NIS and CCE countries are interesting in a number of aspects, and as such an appropriate target for the planned programme. First, these countries belong to the group of intermediate risk for cervical cancer with the following recent statistics. In Russia, 11.714, 11.864, and 11.809 new cervical cancer cases recorded in 1993, 1995, and 1996, respectively, with the incidence of 15/10^5. The figures are of similar magnitude in Latvia, with 183-189 new cases (13.3-13.9/10^5), and somewhat lower in Belarus; 783 new cases (5.6/10^5) being registered in 1993 (Dvoirin et al., 1994). Second, there is, as an inheritance from the past, a law (Order of Ministry of Health of the Soviet Union No: 770 from May 30, 1986 "General dispensarisation of population"), whereby screening by PAP smear is legitimated by stating that every woman from 18 years of age has to be examined by cervical smear every year. However, unlike in the former DDR, where mass-screening was efficiently implemented for 15 years, this Order No: 770 never reached a widespread implementation, and needless to say, has been obscured by the recent political and economic reform of NIS. Third, as evident from the rapid increase of a wide variety of infectious diseases (including diphtheria, tuberculosis, toxic coliform, salmonelles, staphylococci with toxic genes, lyme, chlamydia, HBV, HCV and others) withing the region of NIS, there is no doubt about the significant impact played by genital HPV infections in these countries (Pokrovskij, 1997). No official figures are available, however, and the awareness of these infections is at a low level even among the medical profession. Practically no research interest has been focused on HPV in the NIS, so far. Fourth, the basic infrastructure for the detection of genital HPV infections and cervical precancer lesions exists in some centers, but interdiscipline coordination, considerable learning of the basics as well as modern facilities are needed to establish an efficient health care system for cervical cancer and its precursors.

2.2. The General Objective
The general long-term objective of the suggested joint research project utilizing the existing expertise available in the partners of the West, is to create facilities for the partners in the NIS and CCE to independently conduct measures for the early detection, accurate diagnosis and effective treatment of cervical HPV infections and associated cancer precursors. Once available in these centers in the NIS/CCE, they could form the nucleus for a more widespread implementation of this expertise in the other NIS/CCE countries, which should inevitably lead to reduction of morbidity and mortality due to invasive cervical cancer.

2.3. Specific Objectives
While prevention of cervical cancer by eradicating the etiological agent (HPV) by vaccination is not feasible as yet, attempts should be focused on creating a health care system capable
of effectively tracing the women at risk for subsequent development of cervical cancer. The basic facilities necessary to accomplish this include: a) cytodiagnosis by PAP smear, b) colposcope, c) intruments for taking directed punch biopsies, and d) possibilities to institute an adequate treatment for significant lesions.

**Object #1:** With the help from the EC, to establish these facilities in the clinics and cytopathology laboratories of the contractors in the NIS/CCE. This is one of the development components of the proposal. Requirements for equipment are included in Table 1.

**Object #2:** The assessment of the scope of cervical HPV infections and cancer precursors as a health problem in the NIS. This objective comprises the research component of this proposal, and can only be achieved after fulfillment of Object #1, and even then, only by supplementing these newly established clinical facilities with the existing expertise in diagnostic cytology, molecular biology and related techniques of the partners in the West, as subsequently described.

**Subobjects of #2:** The assessment of the HPV/CIN problem necessarily involves a number of specific subobjects, focused on analysis of the key epidemiological parameters of genital HPV and cancer precursors. These include: 1) the prevalence and 2) risk factors of the disease, 3) disease prognostication, 4) modes of transmission, and 5) geographic variation within the NIS/CCE.

**Object #3:** Comparison of the traditional diagnostic tools (cytology, colposcopy, histology) with the molecular detection techniques (HPV typing by hybridization and PCR) in accomplishing Object #2 and its subobjects. This is of central importance while considering the feasibility of this new technology in the health care system to be developed for cervical cancer prevention in the NIS/CCE. In addition to this developmental aspect, this object also serves the purposes of the research component of the project, while applying molecular techniques in the study of epidemiology (i.e., molecular epidemiology).

**Object #4:** Health care system created to control HPV infections and cervical cancer in the NIS/CCE. This object can only be achieved by fulfillment of objects 1 through 3. It represents the final step in this project, which progresses from the creation of the basic facilities for HPV/CIN diagnosis in the NIS/CCE (Object #1), through the optimal utilization of these facilities to establish the scope of the health problem (by studying different target groups)(Object #2 and its Subobjects), and by evaluating the feasibility of the different technologies (Object #3), ending up with the careful cost-benefit analysis to recommendations for a system to control of cervical cancer within the NIS/CCE.

### 3. SIGNIFICANCE OF THE PROPOSED RESEARCH COOPERATION

Many of the health problems not adequately coped during the last few years of SU were inherited as such to the NIS. One of these major problems pertinent to this proposal is the alarming increase of a wide variety of infectious diseases. These include all types of severe infections which have been practically eradicated in the West decades ago (Pokrovskij, 1997). Of particular significance among the infectious diseases are those caused by HPV, an established human tumor virus, proven to be carcinogenic in humans (IARC, 1995). Epidemiological data from many different countries are consistent in that genital HPV infections are the most common STD, with the prevalence figures as high as 2-3% in the general population (Syrjänen et al., 1990b; 1990c). This disease is significantly more frequent in young, sexually
active women, among whom the annual incidence can approach 10% (Syrjänen et al., 1990c). Analysis of large series of asymptomatic males suggests that young men are equally affected (Hippeläinen et al., 1991; 1993; 1994; Syrjänen, 1995a), sharing the risk factors for contracting an HPV infection; young age at the onset of sexual activity, multiple partners, failure to use condom, poor hygiene, smoking, etc. (Munoz et al., 1990; 1992: Kataja et al., 1992).

There is little doubt that the sexual contact is the most important mode of transmission of genital HPV infections in adults (Oriel, 1971; 1981; Syrjänen et al., 1987; de Palo et al., 1988; von Krogh et al., 1989; Gross et al., 1990; 1997; Monsonego, 1990; 1995; Munoz et al., 1990; 1992; Crum et al., 1991; zur Hausen, 1994; IARC 1995; Mindel, 1995). Recently, however, also other possible modes of transmission have been suggested for HPV, including autoinoculation, infection from moist dwellings, contaminated instruments and underwear, as well as a vertical transmission from an infected mother to her newborn baby at delivery or transplacentally (Sedlacek et al., 1989; Ferenczy et al., 1989; Bergeron et al., 1990; Kellokoski et al., 1990a; 1990b; 1992; Fairley et al., 1993; Handley et al., 1993; Pao et al., 1993; Yun et al., 1993; Armbruster-Moraes et al., 1994; Kaye et al., 1994; Pakarian et al., 1994; Downey et al., 1995; Puranen et al., 1996a; 1996b). Of major significance would be the confirmation of the suggested vertical transmission from an infected mother to her newborn baby, because of its widespread implications e.g. to the planned vaccination programmes (Puranen et al., 1996a; 1996b).

So far, almost all HPV research has been done outside the NIS/CCE. Accordingly, there is practically no information about the key epidemiological issues characterizing the scope of the health problem created by these infections in these countries. This also reflects the generally low level of awareness of this important STD among the general population and medical profession.. An indirect indication of the magnitude of the problem are the constantly high incidence rates (10-15/10^5) of invasive cervical cancer, for which HPV is the most important single etiological factor (Syrjänen et al., 1984; 1990; Munoz et al., 1988; Syrjänen, 1989; Beutner et al., 1991; Koutsky, 1991; Koutsky et al., 1988; 1989; Bosch et al., 1989; 1992; 1994; Kiviat et al., 1993; Schiffman, 1994; Schiffman et al., 1993; 1995; Palefsky et al., 1995; Duggan, 1996; Morris et al., 1996; zur Hausen 1996a; 1996b). These figures have remained stable through the 1990’s, despite the 1986 legislation, requiring an annual PAP smear screening of all women older than 18 years. Practice e.g. in Finland has shown that a full implementation of such an organized screening programme even at 5-year intervals has reduced the incidence of cervical cancer into one fourth in less than 30 years (Hakama 1997), and screening of all sexually active women annually would practically eradicate the disease (Franco et al., 1996; 1997). This failure to show any such effects in the NIS/CCE must be an indication of the fact that the health care system is clearly ineffective in controlling cervical precursors and HPV infections. In fact, the number of smears taken at gynecological visits eg. in Latvia has dramatically decreased from 73% in 1985 to 30.8% in 1996.

The design of this project is a stepwise progression through the research component to the development of an effective infrastructure in carefully selected clinics of the NIS/CCE partners, to be implemented more widespread within the NIS/CCE in the future. Once proven its efficacy, which in this case will take a number of years, this health care system could be adopted to other countries outside the NIS/CCE. Because of the fact that invasive cervical cancer is a considerable health burden in many countries of the Western Europe as well, this project would reach an even more widespread European dimension, by adaptation of the system for the local needs in such risk countries. If successfully implemented, such a health care system is a guarantee of a reduced morbidity and mortality due to cervical cancer.
in these countries, as shown by the experience in Finland (Franco et al., 1997; Hakama, 1997).

The main significance of the proposed cooperation will be in the fact the existing but clearly ineffective health care system in the NIS/ will be developed more efficient to cope with this major health problem. This can only be achieved by a joint research project with the expertise from the West and utilizing their existing health care system as a reference (Syrjänen, 1995a; Franco et al., 1997; Hakama, 1997).

4. SCIENTIFIC AND TECHNICAL DESCRIPTION

4.1. General
This study is a combination of a classical population-based, cross-sectional (prevalence) study combined with a prospective cohort study. Thus, during the first year of this project, consecutive series of women attending the participating centers in the NIS/CCE are screened for cervical HPV infections and CIN, and the positive women (=prevalence data, geographic variation) will then comprise the cohort to be prospectively followed-up in these centers up to the end of this 3-year project (=disease prognosis). These clinics also complete the collection of the epidemiological data by questionnaires focused on the known or suspected risk factors of HPV and CIN (=risk factors, transmission). The samples are examined in part by the laboratories of the NIS/CCE partners, and the diagnosis will be confirmed by the partners in the West (Finland, Italy). The obtained samples will be subjected to further examination for HPV using the in situ hybridization (ISH) and PCR techniques (=molecular epidemiology), as well as for the prognostic factors using quantitative image analysis and immunohistochemistry for a variety of tumour markers. These analysis should clarify the scope of this disease problem, by elucidating the key epidemiological parameters of genital HPV and cancer precursors: These data are necessary for a meaningful planning of the measures for disease control in these centers.

4.2. Patients and Study Design
The detailed flow-chart of the patient examination is shown in Figure 1. To assess the different levels of risk for HPV and CIN, the study is focused on two target populations of women in the NIS; 1) those attending the Gyn. Obst. clinics, and 2) those attending the STD clinics. The first 12 months of the project will be used for recruitment of the women with HPV and/or CIN among the consecutive women attending the Gynecological Departments and STD clinics of the NIS/CCE partners. The detection of the disease will be completed through two arms: 1) by PAP smears (ARM I), and 2) by PCR (ARM II)(Figure 1). This enables the comparison of the efficacy, feasibility and cost-effectiveness of the new molecular diagnostic tools (PCR, ISH) with the traditional HPV/CIN detection methods (PAP smear, colposcopy, biopsy).

Accordingly, all patients attending the clinics of the NIS/CCE partners will be subjected to both PAP smears and cervical swabs (for PCR). Following the ARM I, PAP smear can result in a normal finding, abnormality consistent with HPV-NCIN (HPV without CIN), or more severe abnormality consistent with HPV-CIN. The latter are referred to colposcopy automatically. Women with Class II smears (equivalent to ASCUS in the Bethesda Classification), will be controlled by a repeated PAP smear at 6 months. If abnormality persists, referral to colposcopy will be made. On colposcopy, the result is either a normal or an abnormal pattern, the latter being invariably confirmed by directed punch biopsies. The result of the punch biopsy
will be used as the gold standard of HPV/CIN diagnosis, with all grades from HPV-NCIN to invasive carcinoma being expected. The biopsies will be further examined by ISH, PCR and other techniques for prognostic markers.

The PCR ARM II is an alternative route to reach the same diagnoses (Figure 1). Cervical swabs will be analysed for HPV amplification by consensus primers and to be confirmed by type-specific PCR. PCR-negative women will constitute the Control Group for all HPV/CIN women, to be analysed for the epidemiological risk factors by questionnaires. All PCR-positive women will be subjected to colposcopy and biopsy confirmation, identical to ARM I. Depending on the severity of the lesion on biopsy, the women are allocated to two mutually exclusive outcome: 1) prospective follow-up, and 2) prompt treatment. Accordingly, women with low-grade lesions (HPV-NCIN & HPV-CIN I) will be followed-up without treatment, whereas those with high-grade lesions are subjected to treatment by any of the available methods (cryotherapy, laser, conization or LLETZ)(Singer et al., 1994; Mindel, 1995; Gross et al., 1997).

4.3. Methods

4.3.1. Papanicolaou Smears
In each clinic, all consecutive women attending the clinic will be subjected to cervicovaginal Papanicolaou smear, interpreted for HPV and CIN according to conventional methods (Koss et al., 1956; Meisels et al., 1976; Purola et al., 1977; Syrjänen, 1979). The primary interpretation of the smears is taken care by the cytologists in the NIS centers, subjected to quality control by the coordinator.

4.3.2. Colposcopy
Facilities for colposcopy exist in the Gynecological Departments of the NIS/CCE clinics, with some urgent need for modern instruments, as indicated in Table 1. The recent colposcopic atlases will be used as a diagnostic aid in problem cases (Singer et al., 1994).

4.3.3. Directed Punch Biopsy for Assessment of HPV and CIN
Directed punch biopsies will be taken according to routine procedures, fixed in 10% neutral formalin, and processed for light microscopic HE-sections. In HE-sections, light microscopic analysis of the lesion morphology will be completed according to the commonly accepted diagnostic criteria for HPV infections and grade of CIN (Meisels et al., 1976; Purola et al., 1977; Syrjänen 1979; 1984; 1986; 1992; von Krogh et al., 1989; Gross et al., 1990; 1997; Monsonego, 1990; 1995).

4.3.4. Epidemiologic Questionnaire
All women with established HPV infection (and/or CIN) as well as those proven to be HPV DNA negative by PCR (Control Group) will be subjected to detail inquiry concerning the implicated or suspected risk factors. Most importantly, these include questions about the reproductive history and sexual behaviour as well as smoking habits (Kataja et al., 1992; 1993). The form succesfully used in the Kuopio Cohort Study (1981-1998) will be used here as well.

4.3.5. Treatment
All lesions with intermediate (CINII) or high-grade cancer precursor (CIN III) (and all eventual carcinomas) will be treated using one of the available techniques (cryotherapy, LLETZ, conization). Facilities for modern laser should be created in one NIS/CCE center.
4.3.6. Follow-up
The follow-up protocol tested in the Kuopio Cohort Study since 1981 will be applied to conduct the 24-month prospective follow-up for the women whose low-grade lesions are not readily treated. The follow-up includes colposcopy, PAP smear and punch biopsy confirmation (Syrjänen et al., 1985; 1988; Kataja et al., 1992; 1993; Syrjänen, 1996; 1997).

4.3.7. Detection of HPV
The presence of HPV will be confirmed by molecular diagnostic tools, ISH and PCR. These molecular tools (used in ARM II) will be compared with traditional HPV/CIN diagnosis by PAP smears (ARM I). Furthermore, a type-specific analysis of HPV is mandatory in the biopsies taken as specified in Figure 1.

4.3.7.1. In Situ Hybridization (ISH)
All biopsies will be analysed by in situ hybridization (ISH) using both a screening cocktail probe and type-specific probes, as previously described.

4.3.7.2. PCR
According to ARM II, all women attending the clinics during the first 12 months of the project will be subjected to cervical swab for PCR analyses. PCR amplification for HPV is done using consensus primers GP5+/GP6+, followed by type specific primers for specific viral typing, followed by SB hybridization.

4.3.8. Quantitative Image Analysis for Prognostic Factors
The well established prognostic factors include 1) the lesion grade at the diagnosis, and 2) HPV type involved (Syrjänen et al., 1990a; Kataja et al., 1992; 1993; Downey et al., 1994; Franco et al., 1997; Syrjänen, 1997). The diagnostic material available from the women to be prospectively followed-up will enable the assessment of a wide variety of other implicated or suspected prognostic factors for disease progression, using quantitative image analysis. These analyses are made at the Department of Pathology, University of Siena, which belong to the foremost European laboratories with expertise in this special technology.

4.3.8.1. DNA Cytometry
DNA cytometry is a quantitative technique analysing the DNA content and cell-cycle in the Feulgen-stained histological sections using an automatic Leica Q500MC image analysis system (Leica, England), completed according to the Consensus Report of the European Society for Analytical Cellular Pathology (1995), as detailed before (Tosi et al., 1992).

4.3.8.2. Nuclear Morphometry
Using quantitative image analysis, the following morphometric parameters of the nucleus are analysed: 1) nuclear area, 2) perimeter, 3) length, 4) breadth, 5) convex perimeter, 6) roundness, 7) curve length, 8) curve width, 9) aspect ratio 10) convex area, as recently described (Tosi et al., 1988; 1992).

4.3.9. Immunohistochemistry (IHC) for Cellular Differentiation and Prognostic Markers
The most widely used technique includes Streptavidin-Biotin-Peroxidase Complex (ABC) methods, with different commercially available monoclonal antibodies (MAB). Currently, this technology is run by automated machines (available in partners of both Turku and Siena) capable of large-scale staining within a few hours.
4.3.9.1. Cytokeratins (CK)
Cytokeratins (CKs) are a family of intermediate filament proteins composed of at least 20 polypeptides, with a epithelial-specific distribution that is at least partially preserved during the neoplastic transformation. At the same time, additional CKs may be expressed, depending on the degree of differentiation of the neoplastic epithelium. Of particular interest in CIN are 1) CKs of the basal cells (MAb; AE 1-AE 3), 2) low molecular weight (acidic) CKs No. 14, 16, 17, 3) CK 8, 4) CK 13, 5) CK 18, and 6) CK 19, previously analysed in these lesions together with Prof. Tosi’s group (Cintorino et al., 1988; Leonchini et al., 1990).

4.3.9.2. Cell-Cycle Proteins
HPV replication is intimately linked with the normal cell-cycle, with which the virus interferes in a highly complex manner (zur Hausen, 1994; 1996a; 1996b). A large number of these cell-cycle proteins can be immunohistochemically analysed and quantitated, including the following: p53, Rb, p21, bcl-2, PCNA, Ki-67, Ki-S2, cyclin-D. When combined with the HPV typing, some of these data might be of prognostic importance in predicting the disease progression (zur Hausen 1996a; 1996b; Franco et al., 1997).

4.3.9.3. Prognostic Markers
A wide selection of antibodies are commercially available against different cellular proteins, extensively studied as prognostic markers in human malignancies, breast cancer in particular. Their role in genital HPV infections is inadequately studied as yet. These factors are linked with a wide variety of tumour-host relationships, the main interest being focused on the following three: 1) the tumour-matrix interactions, 2) interactions of growth factors and tumour matrix components, and 3) angiogenesis (Brooks et al., 1994). Among all these categories, important (and in HPV/CIN lesions largely unexplored) markers exist, which can be easily analysed by IHC (Porter-Jordan et al., 1994). Thus, in category 1, E-cadherin, -catenin, 72kd and 92kd collagenases, laminin- and collagen receptors, stromelysins, plasminogen activation, cathepsin D, FGF and TGF. In category 2, of particular interest will be the assessment of integrin family, tenascin, fibronectin, laminin, thrombospondin, FGF, TGF- and TGF- , EGF, PDGF, IGF-family. Angiogenesis is of particular importance for tumour invasion, contributed by a complex interplay of factors such as bFGF, VEGF, TGF- , TNF- , IL-6, TIMP-1 (Folkman, 1992; Montesano et al., 1992; Brown et al., 1995).

4.3.10. Apoptosis
Apoptosis is a gene-directed programme of cell death implicated in cell turnover in normal adult tissues (Alison et al., 1992) and it is regulated by a large number of factors as hormones, growth factors and mitogens (Vaux, 1993). The increase in the concentration of ionised calcium in the cytosol has been demonstrated to be the first detectable signal of apoptosis (Carson et al., 1993), while cAMP seems to play an important role as a second messenger (Kizaki et al., 1990). The final common pathway is represented by a peculiar DNA degradation and fragmentation that is morphologically well defined. Apoptosis may be measured by a series of laboratory techniques, including the TUNEL method (Gavrieli et al., 1992). So far, few studies have assessed the correlation between apoptosis and type of HPV (Xu et al., 1995, Isacson et al., 1996). This analysis may help to clarify some mechanisms by which different types of HPV DNA may give rise to lesions with different biological behaviour.

5. ORGANIZATION AND MANAGEMENT

5.1. Partners and Coordinators
This proposal is a joint research project, contributed by three centers in two EC countries
(Finland, Italy), and 5 centers from three NIS/CCE countries (Russia, Belarus, Latvia). The list of partners and their detailed tasks are summarized in Table 1. The setup of this consortium was carefully considered, based on extensive previous co-operation in other projects (Kuopio-Siena, since early 1980’s; Kuopio/Turku-Moscow since the early 1990’s; and centers in the NIS/CCE since 1970’s).

The basic organization is that of a joint research project between EC and NIS/CCE, with the coordination in the West (Kuopio, Finland) and scientific coordination in Russia (Moscow). The proposed coordinator has a previous experience as the coordinator of another EC Project (Contract # TS3*-CT94-0295) with 3 years duration (1995-1997); for the proposed Scientific Coordinator, Dr. Irena P. Shabalova, this is the first time. The coordinator has also gained a close view on the current level of HPV/CIN awareness in Russia as well as the general level of their health care system by multiple visits to RAMS (Moscow) during the 1990’s.

5.2. Management
There are two Phases in the project: 1) Patient recruitment (12 months), and 2) Patient follow-up (the last 24 months). The flow-chart of patient examination, treatment and follow-up is shown in Figure 1. Well defined tasks and working plan has been allocated to each clinic and NIS/CCE laboratory, as summarized in Table 1. It is fully realized, however, that the participating clinics and laboratories within the NIS/CCE have variable facilities and readiness to do the work to which they are committed, and considerable management will be necessary even before the practical work can be started. This comprises the following components: 1) Assessment of the existing material facilities and equipment as well as the shortages thereoff (already done before the setup of the consortium). 2) Early compensation of the existing shortage of necessary material resources and equipment (to be funded from the project). 3) Once at satisfactory level, phase I (Patient Recruitment) can be started. 4) A continuous follow-up of these material facilities, necessary to continue the work as agreed. This will be on responsibility of the scientific coordinator (assessment of the needs) and coordinator (allocation of funds) jointly.

5.3. Coordination
The coordinator has the overall responsibility of controlling that the work allocated to each of the contractors will be adequately done as detailed in Figure 1 and Table 1. The prerequisite for a successful accomplishment of this task is a competent scientific coordinator within the NIS/CCE. Coordination is needed before the start of the work, and during the entire 36-month continuation of the project. This coordination consists of 1) practical tasks, and 2) work at conceptual level. These practical tasks include e.g. the organization of all data recording (by different agreed forms), system for submitting the samples to examination at different partners, central data registry, feedback to partners, and early troubleshooting in case of suboptimal operation of the consortium.

Work at conceptual level should guarantee all the NIS/CCE partners the necessary expertise to conduct their daily work in the project. Because not at adequate level at the moment, the basic concepts of diagnosis, treatment and follow-up of women with HPV/CIN in the NIS/CCE must be updated before Phase I of the project. This will be done by arranging a Practical Training Course (PTC) on HPV and Cervical Carcinogenesis in Moscow (for all partners) before the start of the practical work. The topics to be exhaustively dealt with include the clinical assessment (colposcopy, treatment, follow-up) of the patients as well as the cytdiagnosis, histopathology, molecular detection methods and prognostication of HPV/
CIN. The clinicians working in the Kuopio Cohort Study since 1981 will be used as consultants in the clinical part of this programme, otherwise run by the EC partners. This basic learning is later supplemented with the technology transfer from the EC- to the NIS/CCE partners, aimed to establishment of IHC, ISH and PCR techniques in their laboratories. This PTC is combined with the first Coordinating Meeting (CM), necessary for both the Management (points 1-4, above), and initial Coordination of the entire working plan, time-schedule and flow-chart of the work (Table 1, Figure 1).

During the continuation of the project, coordination at conceptual level is provided by different means, including 1) the quality control of the diagnostic work in NIS/CCE (review of both cytology and histology), 2) feedback given to the NIS/CCE partners, 3) collecting the results, keeping the centralized data registry, analysis of the data, and 4) providing additional practical training (individually or collectively) if proven necessary. In addition, regular CMs are considered necessary for the close monitoring of the project, most likely to be arranged at 6-9 month intervals with all partners, and if necessary, with individual partners as well. To facilitate the overall coordination, some limited funds should also be allocated for improved communication, e.g. telefaxes.

6.EXPECTED RESULTS

This study design permits an extensive analysis of a number of important issues, necessary while completing the OBJECT 4 of this project (see above). Such key issues include the following: 1) How many cases of significant lesions are missed by ARM I protocol? 2) How many unnecessary colposcopies are caused a) by AMR I protocol, and b) by ARM II protocol? 3) What is the optimal diagnostic setup to result in the highest specificity, sensitivity and positive predictive value of HPV/CIN diagnosis? The results to these key questions will then be carefully weighted while developing the most appropriate health care system for the NIS.

With the prospective follow-up of low-grade lesions (HPV-NCIN & HPV-CIN I) without treatment permits the analysis of a wide variety of prognostic factors with potential impact on disease progression (Syrjänen, 1986; 1989; 1992; Syrjänen et al., 1990). The 24-month period allocated for this follow-up (after the 12 months of recruitment period) is optimal for this purpose, and based on the unequivocal data from the Kuopio Cohort Study, indicating that practically all lesions predestined to progression do so during the first two years from the diagnosis (Syrjänen, 1990; 1992; 1995; 1997; Kataja et al., 1992; 1993). These data should also permit the detection of the low-risk patients to be allocated for follow-up only, and thus avoiding the waste of the limited resources for unnecessary treatment. This should have a major impact on the health economy issues in the NIS/CCE countries with limited resources.

Through the research component of the project, the answers to following key questions should be obtained. 1) The magnitude of genital HPV infections, CIN and cervical cancer as a health problem in these countries. 2) Which are the risk groups for this disease within the NIS/CCE countries? 3 ) The optimal (and the minimum) requirements for an appropriate detection of these risk groups by taking into account the local (limited) resources. 4) By combining the research and development component of this project, an effective health care system could be created to the participating NIS/CCE centers, capable of independently diagnosing and treating genital HPV and CIN, with the decreased disease burden due to cervical cancer as the main long-term objective.
LITERATURE CITED


Dvovir VV, Aksel EM, Trapeznikov NN. Cancer incidence and mortality from in Russia and some NIS countries in 1993.


