Center for Cervical Cancer Prevention

CCCP

Scientific Report 2014-2021

Cover: Transmission electron microscopy photograph of HPV197 Virus-Like Particles. Staining Helena Faust. Photo Kjell Hultenby. InDesign Ulla Rudsander. Logotype Emilie Hultin.

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Center for Cervical Cancer Prevention

-translating scientific advances to improved prevention.

The Center for Cervical Cancer Prevention (CCCP) was created by the Karolinska University Hospital in 2014. The main idea was to exploit the research conducted at Karolinska Institutet on basic science of Human Papillomavirus (HPV) into HPV-based prevention. The center includes an international reference laboratory function where sequencing, documenting and storing all new human papillomaviruses are core tasks.

The goal of the CCCP is to contribute to the global elimination of cervical cancer. Major strategies include:

- How to improve cervical screening

- How to increase the participation in screening

- How to make screening more accessible using self-sampling

- How to increase sensitivity/specificity of the screening test using systematic development and exploration of biomarkers suitable for screening.

-Monitoring the effectiveness of vaccination programs using surveillance of whether vaccine-preventable HPV types are eradicated as expected

The vision of the CCCP is to contribute with new knowledge useful for the global elimination of cervical cancer.

Brief facts about CCCP

- A leading contributor to the international development in HPV-based cancer prevention
- More than 25 employees performing clinical and research activities within the Center
- The Center offers services on all aspects of cervical cancer prevention, at both regional, national and international levels
- Key components of CCCP are
    - o the Swedish National Cervical Screening Registry (www.nkcx.se)
    - o one of the internationally largest HPV testing laboratories
    - o a large biobank of cervical samples
    - o research on both cervical screening and HPV vaccination
    - o a national HPV reference laboratory and an international HPV reference center (www.hpvcenter.se)
- Major methods used include next-generation sequencing, HPV-testing and typing, registry-based research, e-science and health economy
- Offers e-learning resources
- Close interaction with healthcare providers and healthcare authorities
- Since 2014 more than 180 research articles have been published

Organization of the center

The Center is located at the Karolinska University Hospital at campus Flemingsberg. The center is headed by Dr. Joakim Dillner, Professor of Infectious Disease Epidemiology. Today (2022), the Center has more than 25 co-workers focusing on cervical cancer screening, biobanking, infectious disease epidemiology, genomics and other aspects of HPV-related research.

"Our vision is to further the knowledge base for effective global elimination of cervical cancer and other HPV-associated cancers."

Major methodologies and resources used

- Whole genome sequencing with bioinformatic analysis of viral sequences
- Viral serology based on pseudovirions
- HPV-based cervical screening
- The Swedish National Quality registry for cervical cancer prevention
- Epidemiology and biostatistics
- Research on IT/eHealth/Bioinformatics

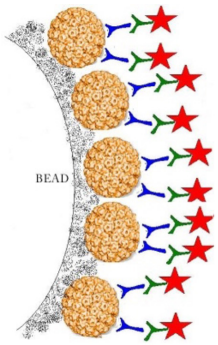


Figure: Validation of multiplexed human papillomavirus serology using pseudovirions bound to heparin-coated beads. Helena Faust, Paul Knekt, Ola Forslund and Joakim Dillner, Department of Medical Microbiology, Malmö University Hospital, Lund University, Malmö, Sweden

Human Papillomavirus Serology using Pseudovirions

HPV serology is essential for vaccinology and epidemiological studies. Our ongoing studies compare the antibody levels induced by different HPV vaccines, where the protective antibody levels needed to prevent high grade lesions after vaccination will be determined. Antibodies directed against the capsid protein L1 that the assay detects have been found to be stable over time.

We use a Luminex-based multiplex assay with HPV pseudovirions as antigens. The pseudovirions are bound to heparin on Luminex beads. Only virions with intact conformation can bind to these beads. Using the multiplex assay, we can analyze simultaneously antibodies against HPV types 3, 4, 5, 6, 8, 11, 15, 16, 18, 31, 32, 33, 35, 38, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 76, 197 and Polyomavirus MCPyV.

International Units (IU) are essential for comparison between different assays and different laboratories. Development of international standard sera used for definition of IU is an ongoing project. Standard sera for HPV 16 and 18 are available, sera for HPV 6, 11, 31, 33, 45, 52 and 58 have been sourced and will be evaluated in an international collaborative study in 2021.

Financial support from Bill and Melinda Gates Foundation is gratefully acknowledged.

Kann H, Lehtinen M, Eriksson T, Surcel HM, Dillner J, Faust H. Sustained Cross-Reactive antibody response after Human Papillomvirus vaccinations: up to 12 years follow-up in the Finnish maternity cohort.

Artemchuk H, Eriksson T, Poljak M, Surcel HM, Dillner J, Lehtinen M, Faust H. Long-term antibody response to Human Papillomavirus Vaccines: Up to 12 years of follow-up in the Finnish maternity cohort. J Infect Dis. 2019 Jan 29;219(4) 582-589.

Faust H, Eklund C, Sukvirach S, Ngamkham J, Dillner J. Sourcing of the WHO human papillomavirus type 18 international standards for HPV antibody levels. J. Clin, Virol. 2016 May;78:89-92

Faust H, Knekt P, Forslund O, Dillner J. Validation of multiplexed human papillomavirus serology using pseudovirions bound to heparin-coated beads. J Gen Virol. 2010 Jull;91: 1840-8

Figure: Supercomputer diagnostics. iStock photo.

Research on IT/eHealth/Bioinformatics

Our unit develops and uses an exhaustive selection of bioinformatic solutions focusing on analysis of the microbes, particularly viruses, at genome and metagenome level. We use user-friendly secure data storage and run analysis pipelines leveraging our big data platform consisting of a Hopsworks Hadoop/Spark cluster. The platform consists of 24 computer nodes, 674 CPU cores, 6 Terabytes of operating memory and 2 Petabytes of storage capacity. Beyond this we also use infrastructural resources at CSC – IT Center for Science Ltd (https://www.csc.fi/) and UPPMAX (Uppsala Multidisciplinary Center for Advanced Computational Science) and local Linux servers. These computing resources enable us to run bioinformatics pipelines at scale and mine for different microorganisms present in a specimen with an increased speedup of at least 11x when using 24 nodes, compared to any sequential analysis pipeline executed on a single node. Furthermore, we have metagenomic bioinformatics capacity for mathematical modeling, deep learning and 'big data' computations to analyze and interpret larger and more complex medical datasets. The combination of large clinical datasets and cutting-edge analytical techniques allows us to better approximate the complex patterns of human exposure interactions that influence our health and well-being.

Our unit also provides informatics services for custom-built softwares for science. For instance, our team has developed an open-source software for invitations, ordering and reporting results in an HPV-based screening project. We also built a customised mobile platform for e-survey data management and analysis, for example used to evaluate the reopening of KI campuses during the COVID-19 pandemic (The KI COVID-19 app).

Research projects

- HPV Serology for the DoRIS trial
- Viruses in human cancers
- International HPV Reference Center
- National HPV Reference Center
- Human Exposome Assessment Platform (HEAP)
- Long-term follow-up of effects of HPV vaccination, including dose reduction
- Risk-based screening for cervical cancer
- The cervical cancer elimination project
- BRIGHT (Biobank- and register-based implementation of gynecological health and screening technologies) and systematic analyses of epigenetics as predictor of major female cancers in the EU project FORECEE
- Formalised collaboration with internationally uniquely large poplation-based cohorts for cancer prevention in Finland
- Epidemiology of SARS-CoV-2 and COVID-19
- Medical diagnostics development

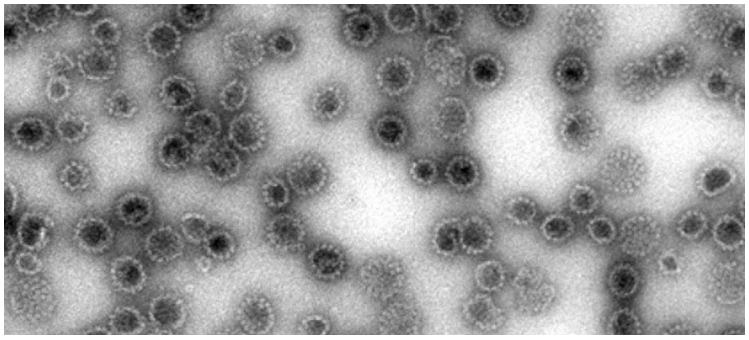


Figure: TEM of antigen used to analyze antibody levels (pseudovirions HPV197). Photo: Kjell Hultenby

HPV Serology for the DoRIS trial

(A Dose Reduction Immunobridging and Safety Study of HPV vaccines)

HPV vaccination is a basis for cervical cancer elimination. The HPV vaccines were originally approved as 3 dose vaccine. Since 2016, 2 doses of the vaccine are recommended for girls younger than 15. The cost for the multi-dose HPV vaccine is a barrier for many countries to start a national vaccination program. The first papers reporting that one dose of the HPV vaccine works just as well appeared now some 5 years ago.

DoRIS is a randomized controlled trial of reduced dose schedule of HPV vaccine of girls age 9-14 years in Mwanza, Tanzania. 900 girls are randomized to receive one, two or three doses of either of the two different vaccines: bivalent targeting HPV 16 and HPV 18 (Cervarix), 9-valent targeting HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 (Gardasil 9). We analyze serum for antibodies directed to HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 at the time of first dose of vaccination, and after 1, 7, 12, 24 and 36 months. Calculation of International Units of antibody levels will be done and compared between the different vaccination arms.

This is the first randomized trial of single dose HPV vaccine in the female target age group.

The study is coordinated by the London School of Hygiene and Tropical Medicine.

Baisley KJ, Whitworth HS, Changalucha J, Pinto L, Dillner J, Kapiga S, de Sanjosé S, Mayaud P, Hayes RJ, Lacey CJ, Watson-Jones D. A dose-reduction HPV vaccine immunobridging trial of two HPV vaccines among adolescent girls in Tanzania (the DoRIS trial) - Study protocol for a randomised controlled trial. Contemp Clin Trials. 2021 Feb;101:106266. doi: 10.1016/j.cct.2021.106266. Epub 2021 Jan 6. PMID: 33421649; PMCID: PMC7970022.

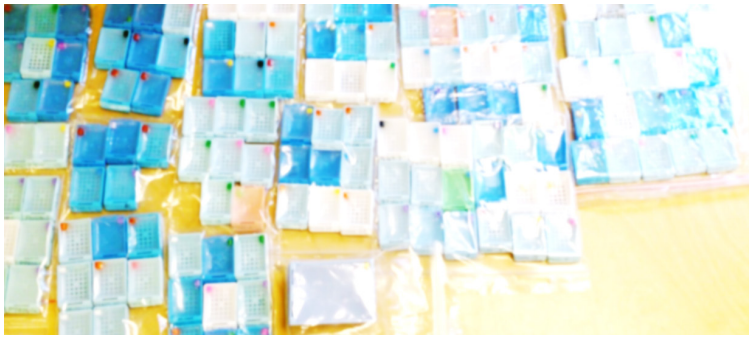


Figure: Formalin-fixed paraffin-embedded tumor blocks. Photo: Emilie Hultin.

Viruses in human cancers

- Focus on infections in cancers among immunosuppressed patients

About 2 million (16%) of the total 13 million new cancer cases that occurred in 2008 are attributable to infections.

Several viruses and bacteria are today known to cause cancer. These infectious agents have a common feature – they establish a persistent infection in their host. The immune system is controlling these agents but the infections can be reactivated in immunosuppressed patients. These patients have an increased risk of cancers, in particular those that are known to be caused by an infection. We are systematically sequencing the tumors that occur in immunosuppressed patients in Sweden.

The patients who have developed cancer after transplantation are identified by linking the Swedish Patient Registry and the Swedish Cancer Registry. The tumor specimens are then requested from the Pathology archives and the tumors sectioned in a contamination-free manner. After extraction of the DNA and RNA, the entire genomic content in the tumors is sequenced and microbial sequences identified through bioinformatics. So far, we have performed whole genome sequencing of about 600 tumors, mostly from skin, lip, cervix, colon and respiratory tract.

Financial support from Swedish research council (Vetenskapsrådet).

Arroyo Mühr LS, Lagheden C, Hassan SS, Kleppe SN, Hultin E, Dillner J. De novo sequence assembly requires bioinformatic checking of chimeric sequences. PloS one 2020 15;8 e0237455.

Arroyo Mühr LS, Bzhalava Z, Hortlund M, Lagheden C, Nordqvist Kleppe S, Bzhalava D, Hultin E, Dillner J. Viruses in cancers among the immunosuppressed. International journal of cancer 2017 141;12 2498-2504

Arroyo Mühr LS, Hortlund M, Bzhalava Z, Nordqvist Kleppe S, Bzhalava D, Hultin E, Dillner J. Viruses in case series of tumors: Consistent presence in different cancers in the same subject. PloS one 2017 12;3 e0172308

Arroyo Mühr LS, Hultin E, Dillner J. Transcription of human papillomaviruses in nonmelanoma skin cancers of the immunosuppressed. Int J Cancer. 2021 Sep 15;149(6):1341-1347. doi: 10.1002/ijc.33683. Epub 2021 May 22. PMID: 33990956.

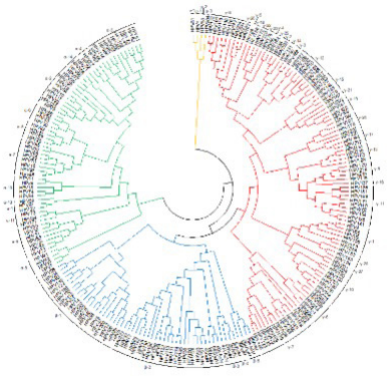


Figure: Phylogenetic tree comprising all official HPV types. Alpha, beta, gamma, mu and nu papillomaviruses are presented in green, blue, red, pink and black colors, respectively. The phylogenetic tree is based on the L1 part of the genome. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993) and analyses were conducted in MEGA7 (Kumar et al., 2016). From hpvcenter.se.

International HPV Reference Center

The International Human Papillomavirus (HPV) Reference Center supports quality and order in HPV research and diagnostics. The Center was originally established at the German Cancer Research Center in Heidelberg in 1985 and was run under the leadership of Dr. Ethel-Michele deVilliers until 2012, year when it was transferred to the Karolinska Institutet, under the leadership of Dr. Joakim Dillner.

The Reference Center is responsible for the HPV taxonomy under the category of species and assigns HPV type numbers to novel HPV types. The established HPV types, currently up to HPV228, belong to 5 different genera: alpha (65 types), beta (54 types), gamma (99 types), mu (3 types) and nu (1 type). The Reference Center maintains a reference clone repository and distributes samples of the reference clones for academic research use.

The International HPV Reference Center also issues international proficiency panels for HPV genotyping, to support reliable and comparable HPV detection services, allowing data to be internationally comparable.

Besides the described activities, the International HPV Reference Center works towards an ambitious program for support and educational services in the HPV field, as well as to promote open science.

All the services and information regarding the center are found at “hpvcenter.se” and are possible thanks to the generous funding by the Bill and Melinda Gates foundation.

Eklund C, Mühr LSA, Lagheden C, Forslund O, Robertsson KD, Dillner J. The 2019 HPV Labnet international proficiency study: Need of global Human Papillomavirus Proficiency Testing. J Clin Virol. 2021 Aug;141:104902. doi: 10.1016/j.jcv.2021.104902. Epub 2021 Jun 19. PMID: 34186414.

Arroyo Mühr LS, Eklund C, Dillner J. Misclassifications in human papillomavirus databases. Virology. 2021 Jun;558:57-66. doi: 10.1016/j.virol.2021.03.002. Epub 2021 Mar 11. PMID: 33730650

Arroyo Muhr LS, Eklund C, Dillner J. Towards quality and order in human papillomavirus research. Virology. 2018;519:74-6.

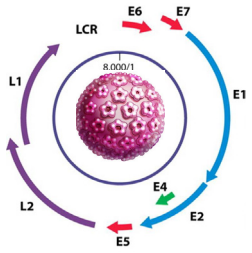


Figure: Genome organization of HPV16.

National HPV Reference Center

The National Human Papillomavirus (HPV) reference laboratory was established by the National Public Health Agency of Sweden in 2017 to support the HPV laboratories in Sweden. The major service provided is on-demand re-analysis of "HPV negative" cervical cancers and high grade precursors. These are re-tested using a broad HPV genotyping test that can identify 37 different HPV types and several variants and after that by whole genome sequencing (Arroyo Mühr et al. Br J Cancer. 2020; Arroyo Mühr et al. J Gen Virol. 2020).

The service is for free within our mission as a reference laboratory.

We analyze both Liquid based cytology, LBC, (ThinPrep and SurePath), and Formalin-Fixed Paraffin-Embedded, FFPE, samples. All samples are handled according to our SOPs.

The National Human Papillomavirus (HPV) reference laboratory also provides other HPV laboratories with samples and plasmids for validation of methods.

The testing strategy is the same as the one we used for systematic HPV genotyping of all cervical cancers diagnosed in Sweden during a ten-year period, 2002-2011. We performed HPV genotyping on all tumors that were released from the regional biobanks (about 3000 tumors) (Lagheden et al. Br J Cancer. 2018). A novel finding was the identification of a distinct subgroup of cervical cancers (about 6% of patients), that have a worse prognosis, have not participated in screening and test HPV-negative. (Lei et al. PLoS Med. 2018).

All the services and information regarding the National Human Papillomavirus (HPV) reference laboratory are found at "hpvcenter.se" and are possible thanks to the generous funding by the Bill and Melinda Gates foundation.

Lagheden, C., Eklund, C., Lamin, H., Nordqvist Kleppe, S., Lei, J., Elfström, K.M., Sundström, K., Andrae, B., Sparén, P., Dillner, J. Nationwide comprehensive human papillomavirus (HPV) genotyping of invasive cervical cancer. Br J Cancer. 118. 1377-1381. 2018. doi: 10.1038/s41416-018-0053-6

Lei, J., Ploner, A., Lagheden, C., Eklund, C., Nordqvist Kleppe, S., Andrae, B., Elfström, K.M., Dillner, J., Sparén, P., Sundström, K. High-risk human papillomavirus status and prognosis in invasive cervical cancer: A nationwide cohort study. PLoS Med. 15. e1002666. 2018. doi: 10.1371/journal.pmed.1002666. eCollection 2018 Oct.

Arroyo Mühr, L.S., Lagheden, C., Lei, J., Eklund, C., Nordqvist Kleppe, S., Sparén, P., Sundström, K., Dillner, J. Deep sequencing detects human papillomavirus (HPV) in cervical cancers negative for HPV by PCR. Br J Cancer. 2020 Oct 6. doi: 10.1038/s41416-020-01111-0. PMID: 33020595.

Arroyo Mühr, L.S., Lagheden, C., Eklund, C., Lei, J., Nordqvist-Kleppe, S., Sparén, P., Sundström, K., Dillner, J. Sequencing detects human papillomavirus in some apparently HPV-negative invasive cervical cancers. J Gen Virol. 101. 265-270. 2020. doi: 10.1099/jgv.0.001374

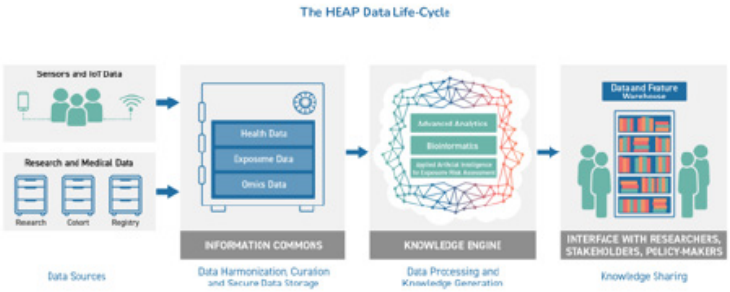


Figure: HEAP data life cycle. Picture from reference below.

Merino Martinez R, Müller H, Negru S, Ormenisan A, Arroyo Mühr LS, Zhang X, Trier Møller F, Clements MS, Kozlakidis Z, Pimenoff VN, Wilkowski B, Boeckhout M, Öhman H, Chong S, Holzinger A, Lehtinen M, van Veen EB, Bała P, Widschwendter M, Dowling J, Törnroos J, Snyder MP, Dillner J. Human exposome assessment platform. Environ Epidemiol. 2021 Dec 3;5(6):e182. doi: 10.1097/EE9.0000000000000182. PMID: 34909561; PMCID: PMC8663864.

Human Exposome Assessment Platform (HEAP)

HEAP (Human Exposome Assessment Platform, https://heap-exposome.eu) is a 5-year funded project part of the European Human Exposome Network (https://www.humanexposome.eu). The project is creating a research and technical platform to assess the impact of the exposome in human health. The HEAP platform integrates management and analysis of heterogeneous sources of exposome data and enables the implementation of state-of-the-art analysis pipelines including bioinformatics, advance statistics, and applied AI. The HEAP consortium is formed by 9 partners from European academic institutions and 2 Small and Medium-sized Emterprises, SME. The project is coordinated by KI under the direction of Prof. Joakim Dillner.

human exposome assessment platform

European Human Exposome NETWORK

European Commission
Horizon 2020
European Union funding
for Research & Innovation

Figure. Vaccination. iStock photo.

Long-term follow-up of effects of HPV vaccination, including dose reduction

In collaboration with the pharmaceutical company Merck & Co. Inc., KI-affiliated CCCP colleagues Karin Sundström, Jiangrong Wang and Sara Nordqvist Kleppe perform register-based observational studies using the Swedish register infrastructure. Dr. Sundström is Principal investigator for a series of projects evaluating the long-term effectiveness and safety of the FUTURE II HPV vaccine trials performed in Sweden. Denmark, Iceland and Norway similarly contribute data and together report data bi-annually to the Food and Drug Administration (FDA) in the USA, and the European Medical Agency (EMA) in the EU, as part of vaccine post-marketing commitment requirements (See Enerly et al, Contemp Clin Trials 2020.)

Dr. Sundström also leads a structured long-term effort in a series of projects aiming to provide real-world data on HPV-related disease incidence in Sweden, as well as continuously evaluate HPV vaccine effectiveness in different dose regimens.

E Enerly, S Berger, S K. Kjær, K Sundström, S Campbell, L Tryggvadóttir, C Munk, M Hortlund, A Joshi, A J. Saah, M Nygård. Use of real-world data for HPV vaccine trial follow-up in the Nordic region, Contemporary Clinical Trials, Volume 92, 2020, 105996, ISSN 1551-7144, https://doi.org/10.1016/j.cct.2020.105996.

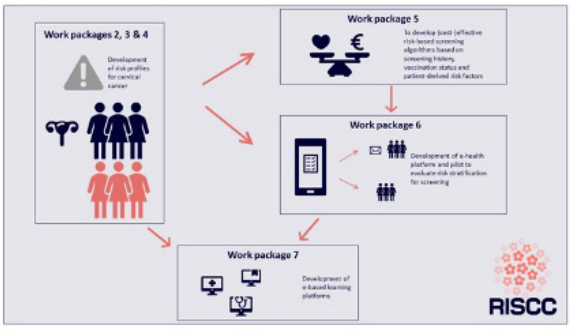


Figure: Work package description in the EU-funded RISCC project. From-RISCC.eu.

Risk-based screening for cervical cancer

RISCC (https://www.riscc-h2020.eu) is a five-year-funded project in the Horizon 2020 framework with 11 European partners dedicated to development of risk-stratified screening of cervical cancer. The project will develop risk profiles from screening history using joint data from several large European HPV screening trials. In addition, risk profiles based on vaccination status will be created for cohorts with varying screening and vaccination coverage, using data from community vaccination trials. RISCC will also build open source e-health applications using risk-based screening algorithms to support implementation in real-life programmes. Karolinska Institutet and Region Stockholm (CCCP) are partners, and the project is coordinated by the company Stichting VUmc in the Netherlands.

European Commission
Horizon 2020
European Union funding
for Research & Innovation

RISCC

Figure: Swedish Cervical Cancer Elimination Hash-tag. From gyncancer.se.

The cervical cancer elimination project

In 2021 we launched a campaign in Region Stockholm/ Gotland inviting all women aged 23-27 to participate in the study "Concomitant HPV-vaccination and screening for fast elimination of HPV infection and cervical cancer" (www.hpvcenter.se). The study investigates if it is possible to accelerate elimination of cervical cancer in Sweden, and is led by CCCP. The elimination project is supported by Region Stockholm, the Swedish Cancer Society, the Swedish Research Council and by the Swedish parliament.

The elimination project was developed by Prof. Joakim Dillner and Dr. Miriam Elfström. It is a further development of the HPV-FASTER concept (1) denoted as the EVEN FASTER concept (2). The HPV reproductive number is >1 (R0) in the age groups that sustain the HPV infection. The project is composed of a campaign to offer concomitant vaccination and HPV screening to women 23-27 years of age. The HPV test will identify the women that are HPV-positive at vaccination, such that they can be followed-up. After the HPV infection has been eliminated, a nationwide one-time screening effort to offer HPV screening to women who may have HPV will be launched.

The HPV vaccination is being performed using vaccination units set up for COVID19 vaccinations, or in conjunction with midwife screening appointments. With iterative rounds of invitations, problem solving and improving the logistics, elimination is expected in Sweden by 2030. The experiences gathered in the project are expected to be useful also in an international perspective, since elimination of cervical cancer is a prioritized health goal globally.

(1) Bosch, F., Robles, C., Díaz, M. et al. HPV-FASTER: broadening the scope for prevention of HPV-related cancer. Nat Rev Clin Oncol 13, 119–132 (2016). https://doi.org/10.1038/nrclinonc.2015.146

(2) https://www.hpvworld.com/articles/the-even-faster-concept-for-cervical-cancer-elimination/

Dillner J, Elfström KM, Baussano I. Prospects for accelerated elimination of cervical cancer. Prev Med. 2021 Dec;153:106827. doi: 10.1016/j.ypmed.2021.106827. Epub 2021 Sep 30. PMID: 34599922.

Vetenskapsrådet

CANCERFONDEN

Swedish Association of Local Authorities and Regions

Region Stockholm

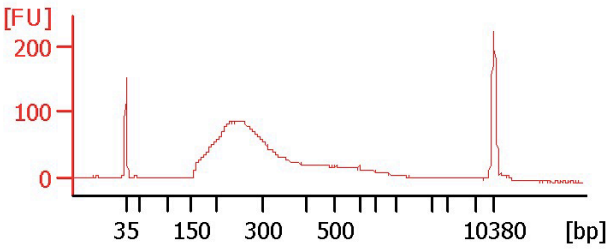


Figure: Bioanalyzer trace of cDNA libraries from 96 FORECEE patient samples analyzed by RNA sequencing.

BRIGHT (Biobank- and register-based implementation of gynecological health and screening technologies)/ FORECEE project

The BRIGHT project was a five-year project for excellence in biomarker research in relation to cervical screening. We used linkage of the Swedish National Cancer Registry and the Stockholm Clinical Cytology Biobank, to measure the association between biomarkers in cervical cells and risk for future gynecological cancer. BRIGHT was funded by the Swedish Foundation for Strategic Research (SSF, www.stratresearch.se) and was a major ongoing effort to utilize frontline sequencing and epigenomics methodology for the improvement of cervical screening. Dr. Dillner was Principal Investigator, Dr. Sundström was Project manager, Dr. Wang was lead analyst and Dr. Helena Andersson provided administrative support.

In synergy with the Horizon 2020-sponsored FORECEE project, we collaborate closely with Professor Martin Widschwendter of University College London, UK, and University of Tirol, Austria. FORECEE aimed to develop a panel of epigenetic markers capable of predicting risk for breast, cervical, endometrial and ovarian cancer development, and utilized the same linkage strategy to identify cases as BRIGHT. Our main technologies used were the Illumina NovaSeq and EPIC array platforms. In BRIGHT, we also liaised with external partner Logical Clocks Inc., who was providing parallelized approaches for the efficient handling of large-scale sequencing data on our 4 peta-byte BRIGHT computer cluster, similarly supported by the SSF.

Widschwendter M, Jones A, Evans I, Reisel D, Dillner J, Sundström K, Steyerberg EW, Vergouwe Y, Wegwarth O, Rebitschek FG, Siebert U, Sroczynski G, de Beaufort ID, Bolt I, Cibula D, Zikan M, Bjørge L, Colombo N, Harbeck N, Dudbridge F, Tasse AM, Knoppers BM, Joly Y, Teschendorff AE, Pashayan N; FORECEE (4C) Consortium. Epigenome-based cancer risk prediction: rationale, opportunities and challenges. Nat Rev Clin Oncol. 2018 May;15(5):292-309. doi: 10.1038/nrclinonc.2018.30. Epub 2018 Feb 27. PMID: 29485132.

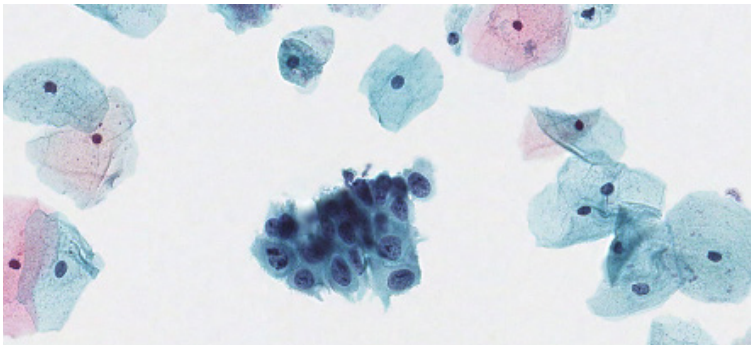


Figure: Cervical Intraepithelial Neoplasia grade 2. Photo Keng-Ling Wallin

Formalised collaboration with uniquely large population-based cohorts for cancer prevention in Finland

Visiting investigator Matti Lehtinen is PI of several very large population-based studies of HPV vaccination and screening in Finland, that have in particular used the nationwide system for archiving of the serum samples taken during maternity care (Finnish Maternity Cohort, FMC). Major current approaches are:

-Identification of protective HPV vaccine-induced antibody levels. Long-term (up to 15 years) follow-up of phase III and IV trials of HPV vaccinated women, where vaccine-induced antibody levels are analysed using state-of-science pseudovirion serology for 15 genital HPV types. After 15 years, there are about 60 cases of potential vaccine "breakthrough" precursor lesions and we are amply powered for the identification of protective vaccine-induced antibody levels. Most notably the natural history of the SIL-lesions is documented with cervical samples taken at the ages of 18, 22, 25 and 28 years and the CCCP lab is genotyping the "breakthrough" cases.

We are also evaluating cellular methylation markers to see whether they could be useful for screening among HPV vaccinated women. We envision that there will be a once-in-a-life-time screening of HPV-vaccinated and/or herd effect protected women and the screening test that we are evaluating for this indication is based on HPV genotyping and triaging with methylation testing.

Gray P, Kann H, Pimenoff VN, Eriksson T, Luostarinen T, Vänskä S, Surcel HM, Faust H, Dillner J, Lehtinen M. Human papillomavirus seroprevalence in pregnant women following gender-neutral and girls-only vaccination programs in Finland: A cross-sectional cohort analysis following a cluster randomized trial. PLoS Med. 2021 Jun 7;18(6):e1003588. doi: 10.1371/journal.pmed.1003588. PMID: 34097688; PMCID: PMC8216524.

Louvanto K, Eriksson T, Gray P, Apter D, Baussano I, Bly A, Harjula K, Heikkilä K, Hokkanen M, Huhtinen L, Ikonen M, Karttunen H, Nummela M, Söderlund-Strand A, Veivo U, Dillner J, Elfström M, Nieminen P, Lehtinen M. Baseline findings and safety of infrequent vs. frequent screening of human papillomavirus vaccinated women. Int J Cancer. 2020 Jul 15;147(2):440-447. doi: 10.1002/ijc.32802. Epub 2019 Dec 16. PMID: 31749143.

Lehtinen M, Lagheden C, Luostarinen T, et al Human papillomavirus vaccine efficacy against invasive, HPV-positive cancers: population-based follow-up of a cluster-randomised trial BMJ Open 2021;11:e050669. doi: 10.1136/bmjopen-2021-050669

Figure: Handling of blood samples for antibody screening. Photo: Ulla Rudsander

Epidemiology of SARS-CoV-2 and COVID-19

To assess the likely burden of COVID-19 infections in the healthcare in Stockholm during the March-June 2020 covid-19 outbreak, we arranged a clinical trial and recruited about 27,000 healthcare workers for SARS-CoV-2 RT- PCR testing and SARS-CoV-2 antibody screening. This included healthcare staff with a variety of professional roles from all healthcare units and premises in Stockholm.

Key findings included :

1. Serum antibodies were accurate predictors of infection and protected against reinfection for about half a year.

2. The amount of virus in the PCR test (the Ct value) was a strong predictor distinguishing past from future COVID sickness.

3. The risk of infection was highly variable between different hospitals, different wards and different professions.

4. About 0.6% of healthy healthcare workers at work were potentially infectious.

5. Employees providing home care services for the elderly were at risk.

Elfström KM, Blomqvist J, Nilsson P, Hober S, Pin E, Månberg A, Pimenoff VN, Arroyo Mühr LS, Lundgren KC, Dillner J. Differences in risk for SARS-CoV-2 infection among healthcare workers. Prev Med Rep. 2021 Dec;24:101518. doi: 10.1016/j.pmedr.2021.101518. Epub 2021 Aug 21. PMID: 34458081; PMCID: PMC8379088.

Dillner J, Elfström KM, Blomqvist J, Eklund C, Lagheden C, Nordqvist-Kleppe S, Hellström C, Olofsson J, Andersson E, Jernbom Falk A, Bergström S, Hultin E, Pin E, Månberg A, Nilsson P, Hedhammar M, Hober S, Mattsson J, Mühr LSA, Conneryd Lundgren K. Antibodies to SARS-CoV-2 and risk of past or future sick leave. Sci Rep. 2021 Mar 4;11(1):5160. doi: 10.1038/s41598-021-84356-w. PMID: 33664279; PMCID: PMC7933367

Dillner J, Elfström KM, Blomqvist J, Engstrand L, Uhlén M, Eklund C, Boulund F, Lagheden C, Hamsten M, Nordqvist-Kleppe S, Seifert M, Hellström C, Olofsson J, Andersson E, Falk AJ, Bergström S, Hultin E, Pin E, Pimenoff VN, Hassan S, Månberg A, Nilsson P, Hedhammar M, Hober S, Mattsson J, Arroyo Mühr LS, Lundgren KC. High Amounts of SARS-CoV-2 Precede Sickness Among Asymptomatic Health Care Workers. J Infect Dis. 2021 Jul 2;224(1):14-20. doi: 10.1093/infdis/jiab099. PMID: 33580261; PMCID: PMC7928785..

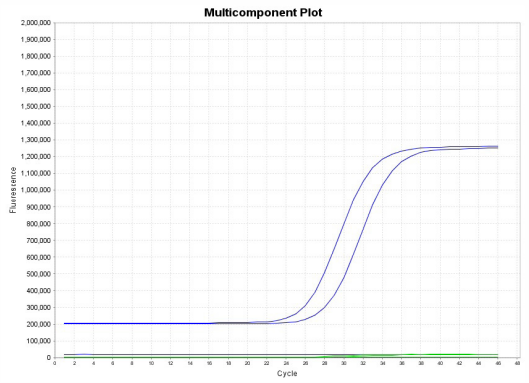


Figure: Chart produced from real-time qPCR.

Medical Diagnostics Development

We receive routine HPV typing samples from pathologists working at Karolinska University Hospital Huddinge and Solna, Södersjukhuset and Danderyds Hospital. Most of the specimens are fine needle biopsies from head and neck tumors which require high-risk HPV typing in very short turnaround time. Sample analysis data and workflow is recorded in the Sympathy database to make it easier for pathologists to establish a final diagnosis. We use an inhouse qPCR test to detect DNA from the E6/E7 region from 13 different HPVs.

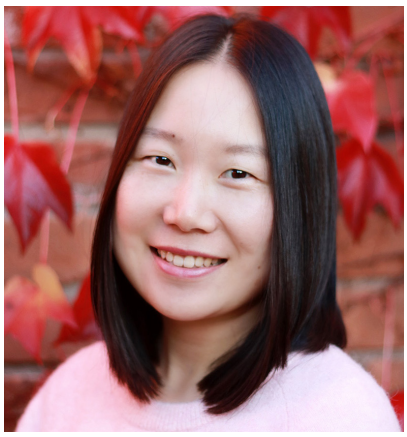
We also perform transcriptome sequencing of solid tumors for pathologists to support rare clinical findings.

The 3 articles with highest attention in society: All by Jiayao Lei et al

Jiayao Lei is post-doctoral researcher at the Center for Cervical Cancer Prevention. She is the lead author of three publications with exceptionally high "altmetric score".

Jiayao Lei studied Preventive Medicine in China and came to Sweden in 2013 for the KI master programme in epidemiology. The main supervisor during her thesis work on cervical cancer prevention was professor Pär Sparén.
The three articles with high altmetric score represent three levels of cancer prevention. Her first paper (PLOS Medicine) concerned prognosis, the second one (British Medical Journal) concerned screening and third one (New England Journal of Medicine) concerned vaccination.
- A lot of work was done before I had the opportunity to analyse the data, e. g. in the HPV genotyping of 3,000 invasive cervical cancer cases diagnosed in Sweden over ten years.
The main finding of the PLOS Medicine article is that absence of high risk HPV types in tumours is associated with worse prognosis of cervical cancer.
-We believe the HPV negative cancers may have contained HPV before and I would be keen to figure out how the virus was lost. Also, it would be interesting to perform a clinical study to see how HPV status in tumours can be used in design of medical treatment.
The British Medical Journal study used the same base of cervical cancers diagnosed over a ten year period as the PLOS Medicine article, but this time the cases were tracked backwards to look at screening history, and the cases were compared with healthy controls. The BMJ article focuses on adenosquamous cell carcinoma and rare histological types of invasive cervical cancer, that were not studied before because of low numbers. The paper shows that cervical screening protects also against these rare cervical cancer types.
-It´s clinically challenging to distinguish the type for those rare cancers so re-reviews by a gyneacologist and a pathologist were important for the validity of the study, Jiayao Lei says.

In the third article, in the New England Journal of Medicine, the nationwide registers in Sweden were linked to study the incidence of invasive cervical cancer in 1,6 million women up to the age of 30 in relation to HPV vaccination. About 30 % of the 1,6 million population had taken the HPV vaccine.
- Because HPV vaccination reduces the risk for HPV infection and precancer, we expected that vaccination would reduce the risk of invasive cervical cancer, Jiayao Lei says.
-We were able to show that HPV vaccination actually prevents cervical cancer.
The altmetric score is measured according to an algorithm that tracks online content and gives weighted contributions to different media sources, such as news outlets, social media and peer review.
Why do you think your articles have attracted so much attention?
-First, I´d like to say it´s a team effort and I feel very grateful for being able to achieve such a good impact in a research field, Jiayoao Lei says. Cervical cancer is the fourth most common cancer among women but it can be eliminated by vaccination and screening, which has high relevance to our daily lives. The NEJM study highlights the significance of HPV vaccination, and it was given a lot of attention and is e.g. one of only 5 key papers cited by the website of the WHO global cervical cancer elimination program.



Jiayao Lei. Photo: Gunilla Sonnebring

Selected publications with societal impact

CCCP has published 184 original scientific articles in the past 8 years. For analysis of the societal impact, we prefer to analyse scientific articles using the Altmetric score that quantifies the attention by media and society. A dozen of the CCCP papers rank in the top 5% of all articles tracked by altmetric score. 10 of them are presented below.

Man I, Vänskä S, Lehtinen M, Bogaards JA. Human Papillomavirus Genotype Replacement: Still Too Early to Tell? J Infect Dis. 2021 Aug 2;224(3):481-491. doi: 10.1093/infdis/jiaa032. PMID: 31985011; PMCID: PMC8328199.

Altmetric score: 53

Here we show in simulations of a transmission model of HPV genotype replacement after HPV vaccination, that the occurrence and timing of type replacement result from a balance between the levels of natural cross-immunity and vaccine-induced cross-protection. If cross-protection is strong enough, it prevents the occurrence of type replacement. If it is weak, the non-vaccine types may first decrease before rebounding into type replacement. The model shows that ten years of follow-up since the introduction of HPV vaccination may be too short to detect type replacement.

Lei, J., Ploner, A., Elfström, K.M., Wang, J., Roth, A., Fang, F., Sundström, K., Dillner, J., Sparén, P. HPV Vaccination and the Risk of Invasive Cervical Cancer. N Engl J Med. 2020 Oct 1;383(14):1340-1348. doi: 10.1056/NEJMoa1917338. PMID: 32997908.

Altmetric score: 5274

Here we show from the study of over 1,6 million Swedish girls and women, aged 10-30 years, that the risk of cervical cancer is much reduced after recieving the quadrivalent HPV vaccine. The protective effect is stronger with those vaccinated before the age of 17 years. The effect is seen not only for high grade cervical lesions, which was shown before, but for invasive cervical cancers, the latter being the ultimate intent of vaccination programs.

Hagman, K., Hedenstierna, M., Gille-Johnson, P., Hammas, B., Grabbe, M., Dillner, J., Ursing, J. SARS-CoV-2 RNA in serum as predictor of severe outcome in COVID-19: a retrospective cohort study [published online ahead of print, 2020 Aug 28]. Clin Infect Dis. 2020;ciaa1285. doi:10.1093/cid/ciaa1285

Altmetric score: 141

Here we investigate the occurrence of SARS Cov-2 RNA in serum from 167 patients admitted to hospital care in Stockholm during 2020. The risk of critical disease or death was 7-fold or 8-fold higher for the 106 patients with virus RNA in their serum.

Vänskä, S., Luostarinen, T., Bausssano, I., Apter, D., Eriksson, T., Natunen, K., Nieminen, P., Paavonen, J., Pimenoff, V.N., Pukkala, E., Söderlund-Strand, A., Dubin, G., Garnett, G., Dillner, J., Lehtinen, M. Vaccination with moderate coverage eradicates oncogenic human papillomaviruses if a gender-neutral strategy is applied. J Infect Dis. 222. 948-956. 2020. doi: 10.1093/infdis/jiaa099.

Altmetric score: 91

Here we show in 33 Finnish trial communities with more than 80,000 resident adolescents that the bivalent HPV vaccine rapidly eradicates oncogenic HPV, except HPV16, with 75% vaccination coverage, comparing HPV prevalence in gender-neutral, girls-only and Hepatitis B vaccinated groups. Also using mathematical transmission models for prediction of HPV16 occurrence, eradication of all high risk types including HPV16 is estimated after 30 years with gender-neutral strategy.

Lei, J., Andrae, B., Ploner, A., Lagheden, C., Eklund, C., Nordqvist Kleppe, S., Wang, J. Fang, F., Dillner, J., Elfström, K.M., Sparén, P. Cervical screening and risk of adenosquamous and rare histological types of invasive cervical carcinoma: population-based nested case-control study. BMJ. 365. l1207. 2019. http://dx.doi.org/10.1136/bmj.l1207

Altmetric score: 86

Here we show that cervical screening is associated with reduced risk for

rare histological types of invasive cervical cancer or adenosquamous cell carcinoma, assessing all over 3,000 cases of invasive cervical cancer in Sweden during 2002-2011. No previous studies have looked at the association between screening and these diagnoses, owing to the rarity of cases.

Lei, J., Ploner, A., Lagheden, C., Eklund, C., Nordqvist Kleppe, S., Andrae, B., Elfström, K.M., Dillner, J., Sparén, P., Sundström, K. High-risk human papillomavirus status and prognosis in invasive cervical cancer: A nationwide cohort study. PLoS Med. 15. e1002666. 2018. doi: 10.1371/journal.pmed.1002666. eCollection 2018 Oct.

Altmetric score: 105

Here we show from genotyping of over 3,000 invasive cervical cancers in Sweden during the years 2002-2011, that women with high risk HPV (13 types) detected in the tumour, which accounted for 81% of cases, had a substantially better prognosis than women with high risk HPV not detected in the tumour. The statistical analysis was performed adjusting for different standard factors relating to prognosis of ICC.

Wang, J., Andrae, B., Sundström, K., Ström, P., Ploner, A., Elfström, K.M., Arnheim-Dahlström, L., Dillner, J., Sparén, P. Risk of invasive cervical cancer after atypical glandular cells in cervical screening: nationwide cohort study. BMJ. 352. i276. 2016. doi: 10.1136/bmj.i276.

Altmetric score: 61

Here we show using registry linkages that for over 14,000 Swedish women diagnosed with atypical glandular cells (AGC) in cervical cytology screening during 20 years, the risk of cervical cancer diagnosis was increased 61 times in the years following the screening occasion, compared to women with normal results in screening. For up to 15 years the risk remained ninefold higher. The study is the first one to show the association between AGC and increased risk of cancer at the population level.

Ronco, G., Dillner, J., Elfström, K.M., Tunesi, S., Snijders, P.J.F., Arbyn, M., Kitchener, H., Segnan, N., Gilham, C., Giorgi-Rossi, P., Berkhof, J., Peto, J., Meijer, C.J.L.M. Efficacy of HPV-based screening for preventing invasive cervical cancer: follow-up of European randomized controlled trials. Lancet. 383. 524-532. 2014.

Altmetric score: 291

Here we follow more than 176,000 women for 6,5 years at HPV-based screening versus cytology based screening of cervical cancer in Sweden, Netherlands, England and Italy. Detection of cervical cancer between screening methods was similar during the first 2,5 years but was significantly lower thereafter in the HPV-based screening group

Herweijer, E., Leval, A., Ploner, A., Eloranta, S., Fridman Simard, J.,Dillner, J., Netterlid, E., Sparén, P., Arnheim-Dahlström, L. Association of varying number of doses of quadrivalent human papillomavirus vaccine with incidence of Condyloma. JAMA. 311. 597-603. 2014.

Altmetric score: 174

Here we show using condyloma as end-point that receipt of 2 quadrivalent doses of HPV vaccine was associated with considerable reduced risk of developing disease compared to 1 dose, and that 3 doses contributed to a relatively small decrease in risk. Over 20,000 cases of condolyma were analysed from a cohort of 1 million women.

Elfström, K.M., Smelov, V., Johansson, A.L., Eklund, C., Nauclér, P., Arnheim-Dahlström, L., Dillner, J. Long term duration of protective effect for HPV negative women: follow-up of primary HPV screening randomised controlled trial. BMJ. 348. g130. 2014.

Altmetric score: 85

Here we show in more than 12,000 women attending organised screening in Sweden, where half of them were enrolled in both HPV-testing and cytology screening, while the other half was enrolled in cytology only, that the increased sensitivity of HPV testing reflects earlier diagnosis rather than overdiagnosis. Screening intervals of 5 years of HPV testing have the same sensitivity as cytology screening intervals of 3 years for HPV negative women.

Doctoral theses

"Human papillomavirus as a target for cancer prevention" 2021 Maria Hortlund

"Vaccine Effectiveness against HPV Infections" 2020 Hanna Kann

"Machine Learning and Data-Parallell Processing for Viral Metagenomics" 2020 Zurab Bzhalava

"Prevention and Prognosis of Cervical Cancer: the interplay of human papillomvirus, vaccination and screening" 2020 Jiayao Lei*,

"Effectiveness and equity of cervical cancer prevention: real-life evidence from organised programmes in Sweden" 2017 Jiangrong Wang*,

"Infections in Skin Cancer" 2016 Sara Arroyo Mühr,

"Optimizing cervical cancer prevention through screening and HPV vaccination" 2015 Miriam Elfström*

"Studies on tumour virus epidemiology" 2014 Davit Bzhalava

"Human Papillomavirsu Test and Vaccination- Impact on Cervical Cancer Screening and Prevention" 2013 Karin Sundström*

* jointly supervised with MEB

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European Union

Swedish Foundation for Strategic Research

Swedish Association of Local Authorities and Regions

Nordic Cancer Union

CIMED

Svenska Läkaresällskapet

Region Stockholm


Center for Cervical Cancer
CCCP Prevention