Uterine Cervical Cancer

Clinical and Therapeutic Perspectives

Samir A. Farghaly
Editor

Springer

Uterine Cervical Cancer

Samir A. Farghaly
Editor

Uterine Cervical Cancer

Clinical and Therapeutic Perspectives

Springer

Editor
Samir A. Farghaly
The Joan and Sanford I. Weill Medical College/Graduate
School of Medical Sciences, The New York Presbyterian Hospital-Weill Cornell
Medical Center, and Sandra and Edward Meyer Cancer Center, Cornell University
New York, NY, USA

ISBN 978-3-030-02700-1 ISBN 978-3-030-02701-8 (eBook)
https://doi.org/10.1007/978-3-030-02701-8

Library of Congress Control Number: 2018963828

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

This book is dedicated to my beloved children Raied and Tamer, and the memory of my mother Amina, and my father Aly who had a great influence on me and my academic and professional medical career. Also, to my sisters Sorya and Nadia, and brother Rafat and their families, and my late siblings, Nabil and Magdy, and their families. In addition to my late nephew, Islam, and my late sister-in-law, Awatif.

Preface

> Uterine cervical cancer is the fourth most common cancer in women, and the seventh overall, with an estimated worldwide 528,000 new cases. A large majority (around 85%) of the global burden occurs in the less developed regions, where it accounts for almost 12% of all female cancers. High-risk regions, with estimated ASRs over 30 per 100,000, include Eastern Africa (42.7), Melanesia (33.3), Southern (31.5) and Middle (30.6) Africa. Rates are lowest in Australia/New Zealand (5.5) and Western Asia (4.4). Cervical cancer remains the most common cancer in women in Eastern and Middle Africa.
>
> There were an estimated 266,000 deaths from cervical cancer worldwide in 2012, accounting for 7.5% of all female cancer deaths. Almost nine out of ten (87%) cervical cancer deaths occur in the less developed regions. Mortality varies 18-fold between the different regions of the world, with rates ranging from less than 2 per 100,000 in Western Asia, Western Europe and Australia/New Zealand to more than 20 per 100,000 in Melanesia (20.6), Middle (22.2) and Eastern (27.6) Africa. [1]

The American Cancer Society's estimates for cervical cancer in the United States of America (USA) for 2018 are about 13,240 new cases of invasive cervical cancer will be diagnosed and about 4170 women will die from cervical cancer [2]. In the USA and Western Hemisphere, cervical precancers are diagnosed far more often than invasive cervical cancer. According to the American Cancer Society, "in the USA, Hispanic women are most likely to get cervical cancer, followed by African-Americans, Asians and Pacific Islanders, and whites. American Indians and Alaskan natives have the lowest risk of cervical cancer in this country" [2]. It has been suggested that declines in cervical intraepithelial neoplasia (CIN2 and CIN3) incidence in the USA are more likely driven by HPV vaccination, introduced in 2006, than by changes in screening or risk behavior.

The purpose of this book is to provide a broad background of several aspects of basic sciences, clinical and therapeutic aspects, and management of uterine cervical cancer. It provides state-of-the-art information on the molecular genetics, biology, and clinical aspects of premalignant lesions of the uterine cervix and uterine cervical cancer. Also, the book chapters provide better understandings of the molecular and cellular events that underlie uterine cervical cancer.

There are 46 contributors to this book who are affiliated with several renowned major academic medical institutions in the USA, the UK, France, Australia, Spain, Greece, Brazil, India, South Africa, and Colombia.

The descriptive and analytical epidemiology of uterine cervical cancer and the role of HPV's infection in the etiology of this disorder are presented in Chap. 1. The strategies for the prevention of uterine cervical cancer which comprises primary, secondary, and tertiary prevention at different stages of the women life are discussed in Chap. 2. The role of several optical technologies in uterine cervical cancer is illustrated in Chap. 3. The program of the screening of uterine cervical cancer in low- and middle-income countries is detailed in Chap. 4. The pathological diagnosis of uterine cervical neoplasia which includes cytopathology, molecular pathology, and surgical pathology is highlighted in Chap. 5. The current information about the prevalence of human papilloma virus (HPV)-associated malignancies in patients with human immunodeficiency virus (HIV) is reported in Chap. 6. The immunological aspects of premalignant conditions of the uterine cervix and the potential efficacy of different immunotherapeutic technologies in treating patients with condition are detailed in Chap. 7. The applicability of sentinel lymph node biopsy in uterine cervical cancer is reported in Chap. 8. The role of fertility-sparing surgery in patients with early-stage uterine cervical cancer due to the trend toward a late childbearing is described in Chap. 9. The current standard and novel surgical treatment of uterine cervical cancer is detailed in Chap. 10. The current management of recurrent and metastatic uterine cervical cancer is discussed in Chap. 11. The role of chemotherapy treatment option for patients with locally advanced and metastatic uterine cervical cancer is highlighted in Chap. 12. The identification of prognostic and predictive biomarkers which allow the knowledge of the subpopulation of patients most likely to respond to radiation therapy is detailed in Chap. 13. The combination of external beam radiotherapy with chemotherapy if fitness allows treating patients with locally advanced and metastatic uterine cervical cancer is discussed in Chap. 14. Finally, negative and positive impact of uterine cervical cancer diagnosis and treatment on the quality of life of these patients are illustrated in Chap. 15.

This book volume is intended for all clinicians and basic medical scientists caring for women with uterine cervical cancer, including attending surgeons and physicians, clinical fellows, and residents in the disciplines of gynecologic oncology, medical oncology, and surgical oncology and also doctoral students and postdoctoral fellows in basic medical sciences.

I would like to thank Margaret Burns, the development editor of this book, and Samantha Lonuzzi, editor at Springer, for their efficiency and valuable help in the process of development, editing, and publishing of this book.

I hope that you find this book very useful and benefit from the extensive experience of the knowledgeable team of contributors who have authored its contents.

New York, NY, USA Samir A. Farghaly

References

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. GLOBOCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC CancerBase No. 11 [Internet]. Lyon: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr. Accessed 28 Aug 2018.
2. https://www.cancer.org/cancer/cervical-cancer/about/key-statistics.html

About the Editors

Samir A. Farghaly is a Professor and Physician/Scientist and national and international expert in Obstetrics and Gynecology at Joan and Sanford I. Weill College of Medicine, Sandra and Edward Meyer Cancer Center and, the New York Presbyterian Hospital/Weill Cornell Medical Center- Cornell University, New York, NY – USA. He received his M.D. from London University and his PhD degree in molecular biology from London University. He was affiliated with major London University teaching hospitals, Columbia University College of Physicians and Surgeons/ Columbia University medical center, New York, NY-USA. He received several national and international clinical and research awards. He has been an invited speaker at several national and international conferences on Women's health, Molecular genetic of female cancers, Gynecological cancer and oncologic radical surgical techniques. He is a member of several national and international societies, organizations, foundations of Women health and Cancer. He is the founder Editor-in Chief of Current Trends in Gynecologic Oncology, and The International journal of Gynecological, Obstetrical and Reproductive Medicine Research journals. Also, he serves as Editor-in- Chief of Enliven: Challenges in Cancer Detection and Therapy Journal and Journal of Reproductive Medicine, Gynecology& Obstetrics. He acts as Senior Editor/ Editor and member of editorial boards, editorial advisory boards of (18) international medical journals on Gynecological Cancers, Gene expression &Therapy, Women's Health and Gynecology. He acted as guest editors of (4) special issues of international medical journals on oncology, Gynecology and gene therapy. He is a reviewer for several medical journals on Obstetrics & Gynecology, molecular Genetics and therapy, Oncology, and Surgery. He has published 105 articles in reputed peer review journals. He has written several book chapters, and is an author and editor of (2) books on ovarian cancer published in 2012, and the third one published in Nov. 2013. The fourth book on endometrial cancer was published in January 2015. The fifth book on recent advances in diagnosis and management Gynecologic cancers was published in March 2016, and the sixth book on ovarian cancer immunotherapy will be published in August 2018. The seventh book on uterine cervical cancer will be published in 2019. The eight's book on ovarian cancer will be published in 2019. The ninth's book on endometrial cancer will be published in 2019.

Contents

Contributors

Yaa Achampong Department of Women's Health, University College London Hospital, London, UK

Georgios Androutsopoulos Department of Obstetrics and Gynaecology, University of Patras, Rion, Achaia, Greece

Peter Bannister Department of Primary Care and Public Health, Brighton and Sussex Medical School, Brighton, UK

Claudia Blancafort Department of Gynecology, Hospital Universitari Quiron-Dexeus, Barcelona, Spain

Stergios Boussios Department of Medical Oncology, Ioannina University Hospital, Ioannina, Greece

Joana Froes Bragança Department of Gynecology and Obstetrics, Hospital Dr. José Aristodemo Pinotti, State University of Campinas, Sao Paulo, Brazil

Mariana Canepa Department of Pathology and Laboratory Medicine, Brown University Warren Alpert Medical School, Providence, RI, USA

Shyrly Carrillo Research Group in Cancer Biology, National Cancer Institute, Bogotá, Colombia

Edward Chandy Clinical Oncology Department, Charing Cross Hospital, London, UK

Catherine Louise Cherry Department of Infectious Diseases, Monash University and Alfred Health, Melbourne, VIC, Australia

Burnet Institute, Melbourne, VIC, Australia

Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Amuthachelvi Daniel Department of Medical Physics, Anna University, Chennai, Tamil Nadu, India

Konstantinos Doufekas Department of Gynaecological Oncology, University College London Hospital, London, UK

Gemma Eminowicz Department of Clinical Oncology/Radiotherapy, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, UK

Samir A. Farghaly The Joan and Sanford I. Weill Medical College/Graduate School of Medical Sciences, The New York Presbyterian Hospital-Weill Cornell Medical Center, and Sandra and Edward Meyer Cancer Center, Cornell University, New York, NY, USA

Oscar Gamboa Unit of Analysis, National Cancer Institute, Bogotá, Colombia

Research Group in Radiobiology Clinical, Molecular and Cellular, National Cancer Institute, Bogotá, Colombia

Maria Luisa Garcia-Moliner Department of Pathology and Laboratory Medicine, Brown University Warren Alpert Medical School, Providence, RI, USA

Aristides Kefas Department of Medicine, Ioannina University Medical School, Ioannina, Greece

M. T. King Faculty of Science, School of Psychology, University of Sydney, Sydney, Australia

Faculty of Medicine, Sydney Medical School, Central Clinical School, University of Sydney, Sydney, Australia

Maria Lombarte Gynecologic Oncology Unit, La Paz University Hospital, Madrid, Spain

Nicolas Magné Department of Radiation Oncology, Institut de cancérologie de la Loire-Lucien Neuwirth, Saint-Priest en Jarez, France

Silvia Marín Gynecologic Oncology Unit, La Paz University Hospital, Madrid, Spain

Alejandra Mateos Gynecologic Oncology Unit, La Paz University Hospital, Madrid, Spain

Diana Mayorga Research Group in Radiobiology Clinical, Molecular and Cellular, National Cancer Institute, Bogotá, Colombia

Anjum Memon Department of Primary Care and Public Health, Brighton and Sussex Medical School, Brighton, UK

R. Mercieca-Bebber Faculty of Science, School of Psychology, University of Sydney, Sydney, Australia

Faculty of Medicine, Sydney Medical School, Central Clinical School, University of Sydney, Sydney, Australia

Linda Mileshkin Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

Pablo Moreno-Acosta Research Group in Cancer Biology, National Cancer Institute, Bogotá, Colombia

Research Group in Radiobiology Clinical, Molecular and Cellular, National Cancer Institute, Bogotá, Colombia

Elisa Moreno-Palacios Department of Gynecology, Hospital Universitario La Paz, Madrid, Spain

Raj Naik Department of Gynaecology, Northern Gynaecological Oncology Centre, Queen Elizabeth Hospital, Gateshead, UK

Adeola Olaitan Department of Gynaecological Oncology, University College London Hospital, London, UK

Alexandra Papadaki Department of Medical Oncology, Ioannina University Hospital, Ioannina, Greece

Nimesh R. Patel Department of Pathology and Laboratory Medicine, Brown University Warren Alpert Medical School, Providence, RI, USA

Evangeline Ponnusamy Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

Chloe Rancoule Department of Radiation Oncology, Institut de cancérologie de la Loire-Lucien Neuwirth, Saint-Priest en Jarez, France

Romelie Rieu Department of Clinical Oncology/Radiotherapy, Charing Cross Hospital, Imperial College Healthcare NHS Trust, London, UK

Alfredo Romero-Rojas Group of Pathology Oncology, National Cancer Institute, Bogota, Colombia

C. Rutherford Faculty of Science, School of Psychology, University of Sydney, Sydney, Australia

Wilfred Prasanna Savarimuthu Department of Physics, Madras Christian College, Chennai, Tamil Nadu, India

M. Tait Faculty of Science, School of Psychology, University of Sydney, Sydney, Australia

Konstantina Tatsi Gynaecology Unit, General Hospital "G. Hatzikosta", Ioannina, Greece

Diama Bhadra Vale Department of Gynecology and Obstetrics, Hospital Dr. José Aristodemo Pinotti, State University of Campinas, Sao Paulo, Brazil

Alexis Vallard Department of Radiation Oncology, Institut de cancérologie de la Loire-Lucien Neuwirth, Saint-Priest en Jarez, France

Ignacio Zapardiel Gynecologic Oncology Unit, La Paz University Hospital-IdiPAZ, Madrid, Spain

George Zarkavelis Department of Medical Oncology, Ioannina University Hospital, Ioannina, Greece

Luiz Carlos Zeferino Department of Gynecology and Obstetrics, Hospital Dr. José Aristodemo Pinotti, State University of Campinas, Sao Paulo, Brazil

Ioannis Zerdes Department of Medical Oncology, Ioannina University Hospital, Ioannina, Greece

Epidemiology of Cervical Cancer

1

Anjum Memon and Peter Bannister

Concepts in Cancer Epidemiology

What Is Epidemiology?

Epidemiology is the art and science of understanding the determinants of health and causation and prevention of disease in the population. It underpins public health and clinical medicine and describes the occurrence and distribution of health-related states or events (incidence, prevalence), quantifies the risk of disease (relative risk, attributable risk, odds ratio) and its outcome (prognosis, survival, mortality) and postulates causal mechanisms for disease in populations (aetiology, prevention) [1]. The main function of epidemiology is to provide evidence to guide public health policy and clinical practice to protect, restore and promote health of individuals and populations. Cancer epidemiology is a branch or subspecialty of epidemiology that studies factors influencing the occurrence (i.e. increase or decrease in incidence of a specific cancer) and prevention of neoplastic and preneoplastic diseases and related disorders.

Measuring the Risk or Burden of Cancer

Incidence

Incidence (or incident cases) is a count of *new cases* of cancer in the population during a specified time period. The incidence rate is the number of *new cases* of cancer in a defined population within a specified time period (usually a calendar

A. Memon (✉) · P. Bannister
Department of Primary Care and Public Health, Brighton and Sussex Medical School, Brighton, UK
e-mail: a.memon@bsms.ac.uk; P.Bannister1@uni.bsms.ac.uk

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_1

year), divided by the total number of people in that population. Cancer incidence rates are typically expressed as per 100,000 population [1, 2].

Age-Standardized Incidence (or Mortality) Rate (ASR)

As the risk of cancer increases exponentially with age, the crude incidence rate (which is influenced by the population age structure) cannot be used to evaluate whether the risk or burden of cancer differs between different populations. It is therefore necessary to use ASRs when comparing incidence rates in populations that have different age structures (e.g. the USA and China). The ASR is obtained by applying the (crude) age-specific rates in the observed population to the age-specific population counts (or weights) of a fixed reference (or standard) population. The most commonly used standard population is the *world* (and also *US* and *European*) *standard population* proposed by Sir Richard Doll. Age-standardization controls for the confounding effect of age on cancer incidence and allows direct comparison between different populations.

Cumulative Incidence (or Cumulative Risk)

Cumulative incidence is the probability or risk of developing cancer during a specified period (e.g. lifetime). It measures the number or proportion of people (out of 100 or 1000) who would be expected to develop a particular cancer by the age of 64 (or 74) years if they had the rates of cancer currently observed. Like the ASR, cumulative incidence permits comparisons between populations of different age structures. For example, the cumulative risk (or lifetime risk) of a woman in the USA developing cervical cancer by age 74 is 0.63% (or 1 in 159) probability [3].

Prevalence

Prevalence is the number of *existing cases* of cancer in a defined population at a notional point in time, divided by the total number of people in the population at that time. It is usually expressed as an absolute number of *existing* cases or as the *proportion* (%) of a population that has the disease. For example, the prevalence of cervical cancer can be defined as the number of women in a defined population who have been diagnosed as having the cancer and who are still alive at a given point in time.

- Partial (or limited duration) prevalence is the estimation of the number of cases of cancer diagnosed within 1, 3 and 5 years to indicate the number of patients undergoing initial treatment (cases within 1 year of diagnosis), clinical follow-up (within 3 years) or not considered cured (within 5 years). Patients alive 5 years after the diagnosis of cancer are usually considered cured because, for most cancers, the death rates among such patients are similar to those in the general population.
- Complete prevalence represents the proportion of patients alive on a certain day who previously had a diagnosis of cancer, regardless of how long ago the diagnosis was or if the patient is still under treatment or is considered cured.

Survival

Survival is the proportion (%) of people still alive 1, 3, 5 and 10 years after they have been diagnosed as having cancer. This *observed* survival probability is influenced by mortality both from the cancer itself and from other causes. For this reason, relative survival (%) is usually calculated (ratio of the observed survival in a particular group of patients to the survival expected in a group of people in the general population).

Mortality

Mortality is the number of deaths occurring, and mortality rate is the number of deaths in a defined population within a specified time period (usually a calendar year), divided by the total number of persons in that population. Cancer mortality rates in adults are usually expressed as per 100,000 persons per year. Mortality is the product of the incidence and the fatality of a given cancer, and measures the average risk to the population of dying from a specific cancer within a specified period. Fatality, the complement of per cent survival, is the probability (%) that a cancer patient will die from the disease.

Cancer Screening

Definition – Screening is the presumptive identification (detection) of an unrecognized disease or defect by the application of tests, examinations, or other procedures that can be applied rapidly.

Cancer screening is the testing of apparently healthy volunteers from the general population for the purpose of separating them into high and low probabilities of having a given cancer. The rationale behind cancer screening is that the disease has a natural history (i.e. phases of pathological progression/cellular transformation) that includes a clearly defined preclinical phase with biological characteristics, which allows for detection of the disease in an early (presumably) treatable stage that, in turn, will reduce the risk of future morbidity and improve survival. For example, cytological screening detects preinvasive cervical disease → intervene with treatment → cure or reduce risk of invasive cervical cancer. Randomized controlled trials and both case-control and cohort observational study designs are used to evaluate cancer screening programmes.

Screening test performance – The performance of a screening test is based on its sensitivity, specificity and predictive value (Table 1.1).

- Sensitivity — this is the ability of the test to identify correctly those who *have* the disease (true positives).
- Specificity — this is the ability of the test to identify correctly those who *do not have* the disease (true negatives).
- Predictive value positive (PVP) — this is the proportion of individuals *who test positive* and actually have the disease. PVP is a function of sensitivity, specificity and prevalence of the detectable preclinical phase. A high PVP is essential for a

Table 1.1 Calculation of sensitivity, specificity and predictive value of a screening test

	Disease according to gold standard			
		Present	Absent	Total
Screening test result	Positive	A (True +)	B (False +)	A + B
	Negative	C (False −)	D (True −)	C + D
	Total	A + C	B + D	A + B+ C + D

Sensitivity = A/(A + C) × 100 (%)
Specificity = D/(B + D) × 100 (%)
Positive predictive value = A/(A + B) × 100 (%)
Negative predictive value = D/(C + D) × 100 (%)
Prevalence of disease = A + C/(A + B + C + D) × 100 (%)

successful population-based screening programme (e.g. cervical cancer), whereas a low PVP implies that resources are being wasted on diagnostic follow-ups of false-positive individuals.

- Predictive value negative (PVN) — this is the proportion of individuals *who test negative* and actually do not have the disease.

Descriptive Epidemiology of Cervical Cancer

Global Burden: Incidence and Mortality

Worldwide, cervical cancer is the fourth most common cancer among women, with an estimated 528,000 new cases (7.9% of cancer in women) and 266,000 deaths (7.5% of cancer deaths in women) in the year 2012 and a 5-year prevalence of 1.5 million cases (9% of women with cancer). In contrast with endometrial cancer, which predominantly occurs in developed countries, the large majority (about 85%) of the cases of cervical cancer occur in developing countries, where it accounts for 12% of all cancers in women [4]. The incidence rates of cervical cancer vary substantially between different populations, from a low of 3.6 per 100,000 women in Switzerland to a high of 75.9 per 100,000 in Malawi (over 20-fold difference). The highest rates are observed among populations in sub-Saharan Africa, Melanesia, Latin America and the Caribbean and South-Central and South East Asia. Incidence rates are generally low in developed countries in Europe, North America, Australia/New Zealand, the Middle East, China and Japan (Figs. 1.1 and 1.2).

In the USA, cervical cancer is the 13th most common cancer among women, with an estimated 12,820 new cases (and 4210 deaths) in the year 2017 accounting for around 2% of all cancers in women, with a cumulative risk of 0.63% (1 in 159) by age 74 (Fig. 1.3) [5–8]. There are an estimated 256,078 women currently living in the USA with cervical cancer [5]. In contrast to endometrial cancer, which predominantly occurs in postmenopausal women, cervical cancer is largely a cancer of middle-aged women [9]. In most European and North American populations, the incidence rates of cervical cancer begin to increase at

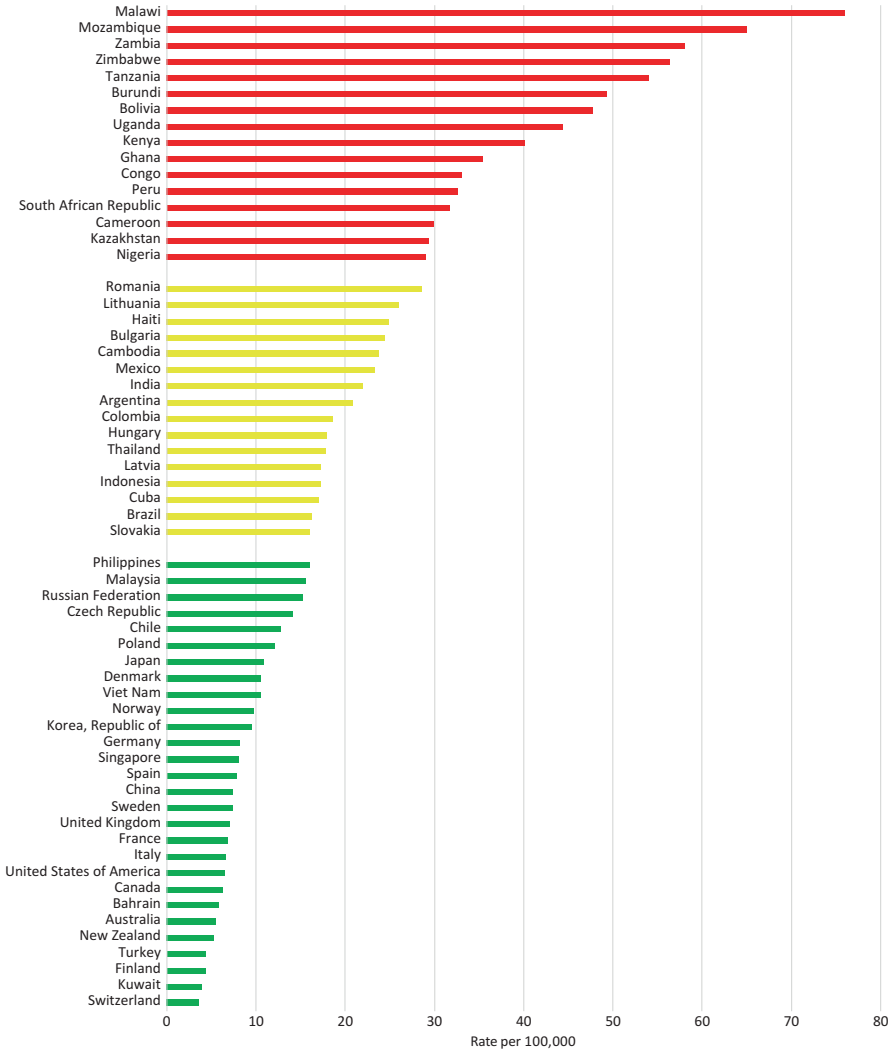

Fig. 1.1 Age-standardized (world standard) average annual incidence rates of cervical cancer in different populations. (From Ferlay [4], with permission)

ages 20–24 years, and thereafter the risk increases rapidly to reach a peak usually around 35–39 years (Fig. 1.4). Cervical cancer is most frequently diagnosed at ages 35–64 years (66% of the cases), and the median age of diagnosis is 49 years (and 58 years at death) [5]. In the USA, the highest incidence rate (9.1/100,000) is observed in Hispanic women followed by 8.7 in black, 7.4 in white and 6.1 in Asian and Pacific Islander women (Fig. 1.4) [5]. The incidence rates of cervical cancer also vary greatly across different states, with the highest incidence in Mississippi (10.4/100,000) and the lowest in Utah (4.59/100,000) [7].

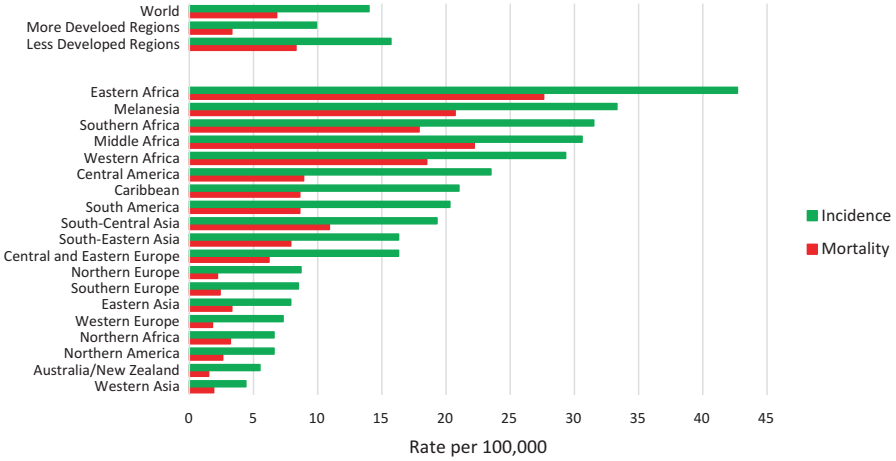

Fig. 1.2 Age-standardized (world standard) incidence and mortality rates as per (10000) of cervical cancer in different populations. (Data from GLOBOCAN [4])

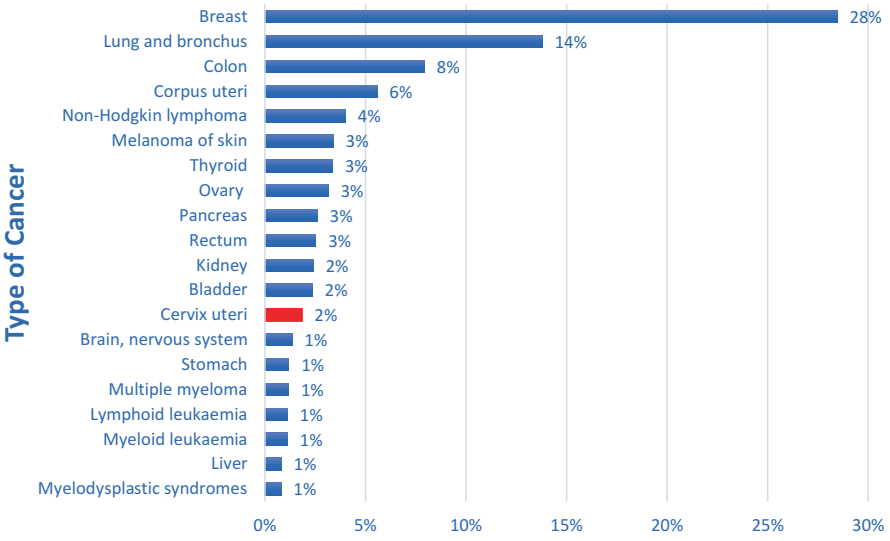

Fig. 1.3 Frequency distribution (%) of the 20 most common cancers in women (all ages and races), USA, 2003–2007. (Data from Forman et al. [3])

Almost nine out of ten (87%) cervical cancer deaths occur in the developing countries. The mortality rates vary substantially between different regions of the world – from less than 2/100,000 in Western Europe to more than 20/100,000 in Africa [4]. In 2014, 890 women in the UK died from cervical cancer (2.8/100,000), accounting for around 1% of all female deaths from cancer. Cervical cancer generally has an excellent prognosis – overall, in the UK, about 63% of women

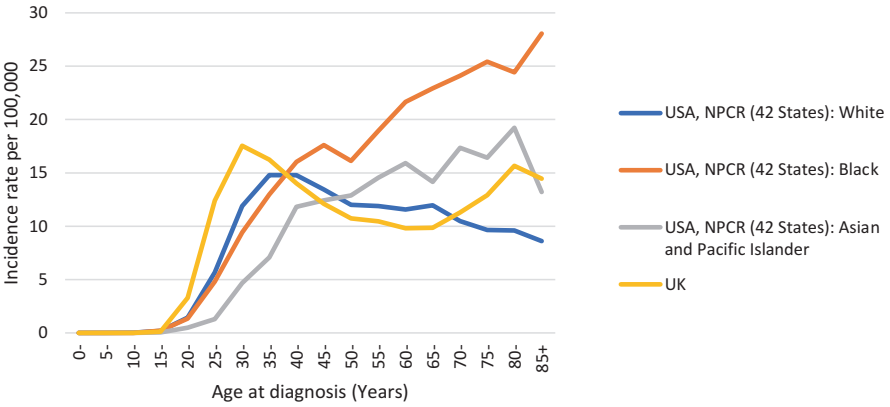

Fig. 1.4 Age-specific incidence rates of cervical cancer in the UK and USA (2003–2007). NPCR, National Programme of Cancer Registries (42 states). (Data from Forman et al. [3])

diagnosed with cervical cancer survive their disease for 10 or more years. When diagnosed at its earliest stage (Stage I), almost all (96%) of the women will survive their disease for 5 or more years, compared to 5-year relative survival of 5% for those diagnosed at Stage IV [10]. In the UK, the 5-year net survival has steadily improved from 51.5% in 1971–1972 to 67.4% in 2010–2011 (an increase of about 31% in the period) [10]. Similarly, in the USA, the overall 5-year relative survival is about 67% and 92% for the localized disease [5]. Due to early diagnosis of precancerous lesions via screening and improvements in treatment, the overall mortality rates of cervical cancer are significantly lower than the incidence. In Western Europe, the cumulative mortality rates are about 4 times lower than the incidence, and in North America cumulative mortality rates are about 2.5 times lower than incidence (Fig. 1.2) [4].

Trends in Incidence and Mortality

Overall, the incidence and mortality from cervical cancer have declined considerably during the past 40 years in Western Europe, North America, Australia/New Zealand, China and Japan (Figs. 1.5 and 1.6). The decline has been attributed to a combination of factors including improved genital hygiene, increased use of condoms, improved treatment modalities, beneficial effects of organized population-based cytological screening programmes for early diagnosis and introduction of the vaccine against HPV infection. In the UK, the age-standardized (European standard) incidence rates of cervical cancer have declined by around 28% since the early 1990s, whereas, in the same period, the mortality rates declined by around 62% [10]. In the USA, the incidence rates of cervical cancer declined by 54% between 1975 and 2014, whereas, in the same period, the mortality rates declined by 59% [5].

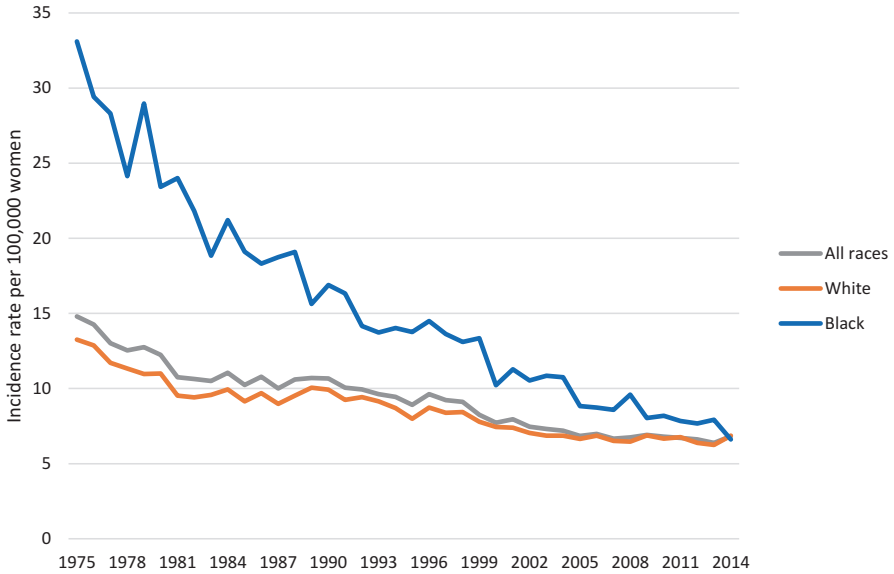

Fig. 1.5 Age-standardized annual incidence rates of cervical cancer in women, USA. (Data from Surveillance, Epidemiology and End Results (SEER) programme [5])

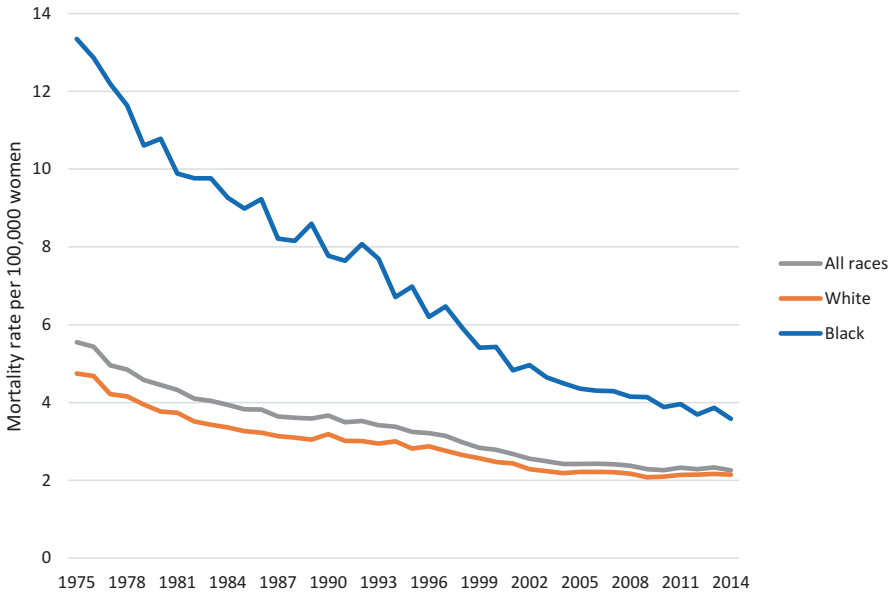

Fig. 1.6 Age-standardized annual mortality rates of cervical cancer in women, USA. (Data from Surveillance, Epidemiology and End Results (SEER) programme [5])

Aetiology of Cervical Cancer

In contrast with endometrial cancer, which is a model of hormonal carcinogenesis, cervical cancer is a model of viral carcinogenesis. The 20-fold variation in age-standardized incidence rates across different populations (Fig. 1.1) point to the role of modifiable factors in the aetiology of cervical cancer – essentially the exposure to, and persistent infection with, the human papillomavirus (HPV) and related cofactors. A persistent infection with an oncogenic HPV type is now recognized as a causal factor for preceding precancerous changes and cervical cancer. However, infection with HPV is extremely common compared with the relatively rare development of cervical cancer. There is compelling evidence that HPV is necessary for cervical carcinogenesis, but infection alone is not sufficient for the cancer to develop. A number of cofactors have been identified as possible modifiers of HPV infection during the developmental stages of cervical cancer, including early sexual debut, increasing number of sexual partners, smoking, long-term oral contraceptive use, high parity, dietary factors, certain human leucocyte antigen (HLA) types and co-infection with other sexually transmitted agents such as *Chlamydia trachomatis*, herpesvirus type 2 and human immunodeficiency virus (HIV) (Fig. 1.7).

Factors Influencing the Risk of Cervical Cancer

Human Papillomavirus (HPV)

The natural history of cervical carcinogenesis as a result of HPV infection is a four-fold process beginning with the virus infecting the metaplastic epithelium of the cervix in the transformation zone [11]. Following initial infection, over 90% of women will go on to clear the virus; however, a small number of women will continue to have viral persistence [11, 12]. This viral persistence can then cause the metaplastic cells to become precancerous cervical intraepithelial neoplasia (CIN) which is graded CIN-I, CIN-II and CIN-III depending upon the extent of the neoplastic change [11]. Invasive cervical cancer develops when these neoplastic cells invade the basement membrane of the cervix [11].

There are many different types of HPV, some of which are low risk and some high risk for developing cervical cancer. HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-45, HPV-52 and HPV-58 are the high-risk HPV types [13–15]. Out of these high-risk types, HPV-16 and HPV-18 are accountable for about 70% of cervical cancers, and 32% of people with an HPV infection are infected with these phenotypes [11].

In order for HPV transmission to occur, genital contact is required with an infected partner [16]. In the USA, HPV is the most common sexually transmitted infection, and there is a strong correlation between HPV infection/persistence and the number of lifetime sexual partners and sexual partners in the past year [16, 17]. The prevalence of HPV is greatest in women aged 20–24 (27.4%), with an increasing prevalence from the age of 14–24 and then a gradual decrease from the age of 25–59 [18]. It is thought that HPV infection/persistence is most common in younger women due to lack of previous exposure and therefore not having developed an

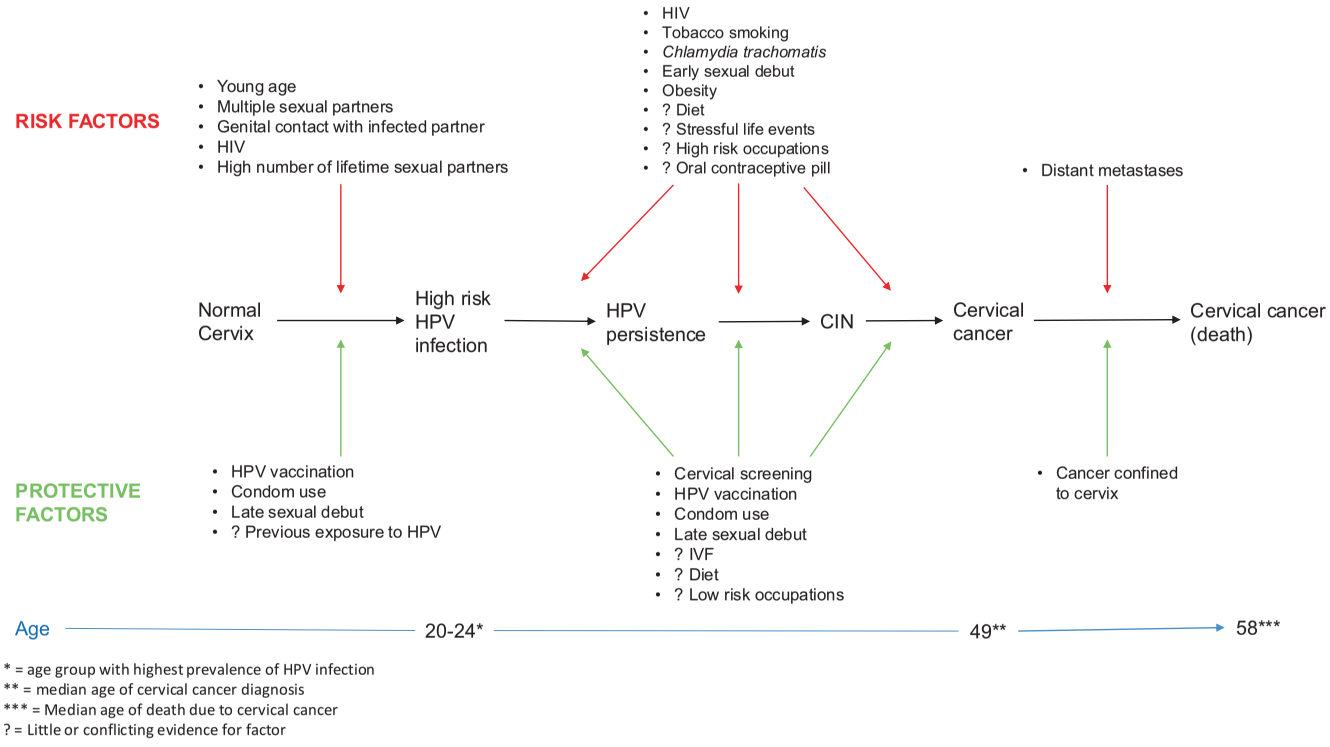

Fig. 1.7 The natural history of cervical cancer

immune response to the virus [16]. Not only does HPV account for almost all cases of cervical cancers; the virus is also associated globally with 113,000 cancers at other anatomical sites, for example, cancers of the vulva, vagina, penis and oropharynx [13, 19].

The prevalence (all ages combined) of HPV differs greatly between populations across the world. The highest prevalence is observed in Africa (22.1%) and lowest in Asia (8.0%) [20, 21]. The overall prevalence of HPV in North America and Europe is 11.3% and 8.1%, respectively [21]. The prevalence of HPV coincides with the incidence rates of cervical cancer in different populations (Fig. 1.1).

Tobacco Smoking

There is convincing epidemiological evidence that tobacco smoking is an independent risk factor for cervical cancer [22, 23]. In the two large collaborative studies on cervical cancer, there was an approximate doubling in risk among current smokers compared to never smokers; and this risk was further increased with younger age at starting smoking and the number of cigarettes smoked per day. In these studies, the effect of smoking appeared to be limited to squamous cell carcinoma of the cervix. In recent studies, an increased risk of cervical cancer has also been reported for women exposed to passive smoking [22, 24, 25]. It has been suggested that cigarette smoking may promote carcinogenicity by affecting local cell-mediated immune response, inducing genetic damage and causing localized immune suppression which may promote HPV persistence [26, 27].

Co-infection with Human Immunodeficiency Virus (HIV)

HIV increases the risk of developing CIN and invasive cervical cancer in the presence of HPV [28]. The prevalence of HPV is greater in HIV-positive people than HIV-negative people (37.2% vs 13.7%, respectively) [29]. Furthermore, persistent infection with HPV-16 or HPV-18 is relatively more common in HIV- positive people compared to those who are HIV-negative (20% vs 3%) [30]. However, despite treatment of HIV with antiretroviral therapy, the risk of developing cervical cancer remains substantially higher than in the HIV-negative population [31]. It is believed that there is a synergistic interaction between HIV and oncogenic HPV-16 – HIV infection compromises the immune system and predisposes sexually active women to co-infection by HPV-16 and its persistence [32].

Co-infection with Chlamydia Trachomatis

Co-infection of HPV with *Chlamydia trachomatis* has been associated with an increased risk of developing squamous cell carcinoma of the cervix in several studies [33–37]. In a pooled analysis of the International Agency for Research on Cancer (IARC) multicentred case-control studies, there was a twofold increased risk in HPV DNA-positive women who were also C. *trachomatis* seropositive compared to those who were seronegative [34]. It has been hypothesized that concomitant genital

infections may induce chronic irritation/inflammation of the cervix which could promote HPV-related oncogenic processes.

Reproductive Factors

Currently there is a good epidemiological evidence to support an association between multiparity and invasive cervical cancer (also CIN and carcinoma in situ), controlling for HPV status or other potential reproductive and sexual behaviour variables [25]. Most studies in populations where multiparity is common have reported an increased risk of cervical cancer among both HPV-positive and HPV-negative women [38]. Several hypotheses have been suggested to explain possible biological mechanisms that may influence the risk, including hormonal, nutritional and immunological changes during pregnancy and/or trauma to the cervix that occurs during parturition.

Sexual Behaviour

It has long been recognized that sexual behaviour played an important role in the aetiology of cervical cancer. It is now well established that an early age at first intercourse and increased number of lifetime sexual partners are associated with an increased risk of cervical cancer and its precursor lesions [25]. As would be expected, the use of condoms is associated with a decreased risk of HPV infection and persistence. In a recent study that demonstrated a protective effect of condom use, the incidence rates of both genital HPV infection and cervical intraepithelial lesions were reduced in condom users compared to nonusers [39, 40].

Obesity

There is some evidence to suggest that obesity, particularly weight gain since age 18, may be a risk factor for adenocarcinoma of the cervix [41]. It has been difficult to assess this association due to a large number of potential confounding factors (i.e. HPV, sexual behaviour, hormonal factors). Similarly, it has been difficult to assess the association with physical activity – some studies have demonstrated a protective effect with moderate to high physical activity [42].

Diet

It is plausible that certain foods and nutrients could have a protective effect against the development of cervical cancer. There is some evidence to suggest that high dietary consumption of carotenoids, retinol, vitamins C and E, folate and fruits and vegetables may reduce the risk of CIN and cervical cancer [43].

Prevention of Cervical Cancer

Cervical cancer is one of the most preventable forms of cancer on a global scale. Prevention efforts include increased public awareness about sexually transmitted infections, early detection of precursor lesions by regular cytological

screening, HPV testing and the recently developed vaccine against certain high-risk types of HPV. Cervical cancer screening in the form of cytology (Pap test) and HPV test (to detect the DNA or RNA of HPV) substantially reduces the lifetime risk of developing cervical cancer [44]. The US Preventive Services Task Force recommends that women aged 21–29 years should be screened every 3 years with cytology and women aged 30–65 years should be screened every 5 years with cytology + HPV test or every 3 years with cytology [7]. In the population-based cervical screening programme in the UK, all women aged 25–49 are invited for screening every 3 years; and women aged 50–64 years are invited for screening every 5 years [45]. The cytological screening programme has been highly effective in reducing both the incidence and mortality from invasive cervical cancer [46]. It is estimated that cervical screening is currently preventing 70% of cervical cancer deaths in the UK, and if all women attended their cervical screening appointment, appropriately 83% of cervical cancer deaths could be prevented [46]. There have also been developments in the use of first-void urine self-sampling as an alternative to physician-led cervical screening which may be an alternative option for women who do not attend cervical screening appointments or live in developing countries with no formal cervical screening programme [47, 48].

Prophylactic vaccines against HPV currently available include monovalent (HPV-16), bivalent (HPV-16 and HPV-18) and quadrivalent (HPV-6, HPV-11, HPV-16 and HPV-18) virus-like particle vaccines. In clinical trials, these vaccines have shown excellent safety and nearly 100% efficacy in preventing persistent infections and precancerous lesions due to HPV-16 and HPV-18. In the UK, all girls aged 12–13 years are offered HPV quadrivalent vaccine as part of the childhood immunization programme [45]. In the USA, HPV vaccination is recommended for girls and boys aged 11–12 years and young women through age 26 and young men through age 21 [7]. It has been estimated that almost all the cases of cervical cancer can be prevented by changes in lifestyle and risk factor modification.

Conclusion

Worldwide, cervical cancer is the fourth most common cancer among women – it accounts for an estimated half million new cases and quarter million cancer deaths in women each year. It is a cancer of the developing world (85% of all cases) and predominantly occurs in middle-aged women. In most European and North American countries, there has been a considerable decline in the incidence and mortality from cervical cancer during the past 40 years. This decline is attributed to a combination of factors including improved genital hygiene, increased use of condoms, improved treatment modalities, beneficial effects of organized population-based cervical screening programmes for early detection/diagnosis and introduction of the vaccines against HPV.

A persistent infection with an oncogenic HPV type is now recognized as a causal factor in almost all cases of cervical cancer. Although HPV is considered necessary for cervical carcinogenesis, infection with HPV alone is not always sufficient for the malignant transformation. A number of cofactors have been identified as possible modifiers of HPV during the development of cervical cancer, including tobacco smoking, multiparity, oral contraceptive use and *Chlamydia trachomatis* infection. As one of the leading causes of cancer among women worldwide, cervical cancer is an important public health problem, particularly in developing countries – 85% of all cases and 87% cervical cancer deaths occur in developing countries. It is now believed that a combination of efforts including health education about transmission of HPV, early detection of precursor lesions via regular screening and population-based vaccination programmes could substantially reduce the burden of cervical cancer and make it the most preventable forms of cancer on a global scale.

References

1. Memon A. Epidemiological understanding: an overview of basic concepts and study designs. In: Pencheon D, et al., editors. Oxford handbook of public health practice. 2nd ed. Oxford: Oxford University Press; 2006. p. 100–11.
2. Memon A. Epidemiology of gynaecological cancers. In: Shafi M, et al., editors. Gynaecological oncology. Cambridge: Cambridge University Press; 2010. p. 1–13.
3. Forman D, Bray F, Vrewster D, et al. Cancer incidence in five continents, Vol X (electronic version) Lyon, IARC. 2013. Available from: http://ci5.iarc.fr/CI5-X
4. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, cancer incidence and mortality worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr
5. The Surveillance, Epidemiology, and End Results (SEER) Program. Available from: http://seer.cancer.gov
6. National Cancer Institute. Cervical cancer. 2014. Available from: http://www.cancer.gov/cancertopics/types/cervical
7. Centers for Disease Control and Prevention (CDC), National Programme of Cancer Registries (NPCR). Available from: http://apps.nccd.cdc.gov/uscs and http://www.cdc.gov/cancer/npcr
8. Ryerson A, Eheman C, Altekruse S, et al. Annual report to the nation on the status of cancer, 1975–2012, featuring the increasing incidence of liver cancer. Cancer. 2016;122(9):1312–37.
9. Ylitalo N, Stuver S, Adami H. Cervical cancer. In: Hans-Olov A, et al., editors. Textbook of cancer epidemiology. 2nd ed. New York: Oxford University Press; 2008. p. 446–67.
10. Cancer Research UK. Available from: http://info.cancerresearchuk.org
11. Schiffman M, Castle P, Jeronimo J, et al. Human papillomavirus and cervical cancer. Lancet. 2007;370(9590):890–907.
12. Castle P, Rodriguez A, Burk R, et al. Short term persistence of human papillomavirus and risk of cervical precancer and cancer: population based cohort study. BMJ. 2009;339:b2569.
13. Crosbie E, Einstein M, Franceschi S, et al. Human papillomavirus and cervical cancer. Lancet. 2013;382(9895):889–99.
14. Guan P, Howell-Jones R, Li N, et al. Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. Int J Cancer. 2012;131(10):2349–59.
15. Li N, Franceschi S, Howell-Jones R, et al. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication. Int J Cancer. 2011;128(4):927–35.

16. Cox J. The development of cervical cancer and its precursors: what is the role of human papillomavirus infection? Curr Opin Obstet Gynecol. 2006;18(suppl.1):S5–S13.
17. Sellors J, Karwalajtys T, Kaczorowski J, et al. Incidence, clearance and predictors of human papillomavirus infection in women. CMAJ. 2003;168(4):421–5.
18. Dunne E, Unger E, Stenberg M, et al. Prevalence of HPV infection among females in the United States. JAMA. 2007;297(8):813–9.
19. Plummer M, de Martel C, Vignat J, et al. Global burden of cancers attributable to infections in 2012: a synthetic analysis. Lancet Glob Health. 2016;4(9):609–16.
20. Franceschi S, Herrero R, Clifford G, et al. Variations in the age-specific curves of human papillomavirus prevalence in women worldwide. Int J Cancer. 2006;119(11):2677–84.
21. De Sanjose S, Diaz M, Castellsague X, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis. 2007;7(7):453–9.
22. International Collaboration of Epidemiological Studies of Cervical Cancer. Carcinoma of the cervix and tobacco smoking: collaborative reanalysis of individual data on 13,541 women with carcinoma of the cervix and 23,017 women without carcinoma of the cervix from 23 epidemiological studies. Int J Cancer. 2006; 118 6 :1481–95.
23. Kapeu A, Luostarinen T, Jellum E, et al. Is smoking an independent risk factor for invasive cervical cancer? A nested case-control study within Nordic biobanks. Am J Epidemiol. 2008;169(4):480–8.
24. Plummer M, Herrero R, Franceschi S, et al. Smoking and cervical cancer: pooled analysis of the IARC multi-centric case-control study. Cancer Causes Control. 2003;14(9):805–14.
25. Schiffman M, Brinton L. The epidemiology of cervical carcinogenesis. Cancer. 1995;76(suppl.10):1888–901.
26. Palefsky J, Holly E. Molecular virology and epidemiology of human papillomavirus and cervical cancer. Cancer Epidemiol Biomark Prev. 1995;4(4):415–28.
27. Burger M, Hollema H, Gouw A, et al. Cigarette smoking and human papillomavirus in patients with reported cervical cytological abnormality. BMJ. 1993;306(6880):749–52.
28. International Agency for Research on Cancer. Human papillomavirus. IARC monographs. 2012; 100b:255–313.
29. De Vuyst H, Gichangi P, Estambale B, et al. Human papillomavirus types in women with invasive cervical carcinoma by HIV status in Kenya. Int J Cancer. 2008;122(1):244–6.
30. Sun X, Kuhn L, Ellerbrock T, et al. Human papillomavirus infection in women infected with the human immunodeficiency virus. N Engl J Med. 1997;337(19):1343–9.
31. Rohner E, Sengayi M, Goeieman B, et al. Cervical cancer risk and impact of pap-based screening in HIV-positive women on antiretroviral therapy in Johannesburg, South Africa. Int J Cancer. 2017;141(3):488–96.
32. Strickler H, Palefsky J, Shah K, et al. Human papillomavirus type 16 and immune status in human immunodeficiency virus-seropositive women. J Natl Cancer Inst. 2003;95(14):1062–71.
33. Dahlstrom L, Andersson K, Luostarinen T, et al. Prospective seroepidemiologic study of human papillomavirus and other risk factors in cervical cancer. Cancer Epidemiol Biomark Prev. 2011;20(12):2541–50.
34. Smith J, Bosetti C, Munoz N, et al. Chlamydia trachomatis and invasive cervical cancer: a pooled analysis of the IARC multicentric case-control study. Int J Cancer. 2004;111(3):431–9.
35. Smith J, Munoz N, Herrero R, et al. Evidence for chlamydia trachomatis as a human papillomavirus cofactor in the etiology of invasive cervical cancer in Brazil and the Philippines. J Infect Dis. 2002;185m(3):324–31.
36. Wallin K, Wiklund F, Luostarinen T, et al. A population-based prospective study of chlamydia trachomatis infection and cervical carcinoma. Int J Cancer. 2002;101(4):371–4.
37. Koskela P, Anttila T, Bjorge T, et al. Chlamydia trachomatis infection as a risk factor for invasive cervical cancer. Int J Cancer. 2000;85(1):35–9.
38. Munoz N, Franceschi S, Bosetti C, et al. Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. Lancet. 2002;359(9312):1093–101.

39. Silins I, Ryd W, Strand A, et al. Chlamydia trachomatis infection and persistence of human papillomavirus. Int J Cancer. 2005;116(1):110–5.
40. Winer R, Hughes J, Feng Q, et al. Condom use and the risk of genital human papillomavirus infection in young women. N Engl J Med. 2006;354(25):2645–54.
41. Ursin G, Pike M, Preston-Martin S, et al. Sexual, reproductive and other risk factors for adenocarcinoma of the cervix: results from a population-based case-control study (California, United States). Cancer Causes Control. 1996;7(3):391–401.
42. Lee J, So K, Piyathilake C, et al. Mild obesity, physical activity, calorie intake, and the risks of cervical intraepithelial neoplasia and cervical cancer. PLoS One. 2013;8(6):e66555.
43. Garcia-Closas R, Castellsague X, Bosch X, et al. The role of diet and nutrition in cervical carcinogenesis: a review of recent evidence. Int J Cancer. 2005;117(4):629–37.
44. Peirson L, Fitzpatrick-Lewis D, Ciliska D, et al. Screening for cervical cancer: a systematic review and meta-analysis. Syst Rev. 2013;2:35.
45. NHS Choices. Available from: http://www.nhs.uk/pages/home.aspx
46. Landy R, Pesola F, Castanon A, et al. Impact of cervical screening on cervical cancer mortality: estimation using stage-specific results from a nested case-control study. Br J Cancer. 2016;115(9):1140–6.
47. Leeman A, del Pino M, Molijn A, et al. HPV testing in first-void urine provides sensitivity for CIN2+detection comparable with a smear taken by a clinician or a brush-based self-sample: cross-sectional data from a triage population. BJOG. 2017;124(9):1356–63.
48. Blake D, Crosbie E, Kitson S. Urinary HPV testing may offer hope for cervical screening non-attenders. BJOG. 2017;124(9):1364.

Prevention of Cervical Cancer

2

Konstantinos Doufekas, Yaa Achampong, and Adeola Olaitan

Cervical cancer presents an important global health challenge. It is the fourth most common cancer in women after breast, colorectal, and lung cancer. It remains a leading cause of death in women worldwide with 530,000 new cases and 275,000 deaths worldwide each year. The majority of cases occur in less well-resourced countries, particularly in sub-Saharan Africa. A fifth of all cases and over 25% of deaths occurred in India. Countries such as Kenya, Uganda, and Nigeria rank high in the mortality stakes [1].

In sub-Saharan Africa, 34.8 new cases of cervical cancer are diagnosed per 100,000 women annually, and 22.5 per 100,000 women die from the disease. These figures compare with 6.6 and 2.5 per 100,000 women, respectively, in North America [2].

Such differences can be explained by lack of access to effective screening or to services that facilitate early detection and treatment. These figures may also be confounded by lack of accurate data due to a dearth of cancer registries in the worst affected countries and because a significant number of women do not seek healthcare or do not receive an accurate diagnosis.

Cervical cancer is a preventable disease, and effective prevention strategies are essential to reduce its burden. Prevention strategies can be broadly divided into primary, secondary, and tertiary prevention.

Primary prevention refers to prophylactic vaccination against the oncogenic types of human papillomavirus (HPV). Secondary prevention is based on cervical screening and has been very successful in high-resource countries. Tertiary prevention is defined by the World Health Organization (WHO) as access to cancer

K. Doufekas (✉) · A. Olaitan
Department of Gynaecological Oncology, University College London Hospital, London, UK
e-mail: konstantinos.doufekas@nhs.net; adeola.olaitan@nhs.net

Y. Achampong
Department of Women's Health, University College London Hospital, London, UK
e-mail: YaaAchaa.Achampong@uclh.nhs.uk

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_2

treatment and management for women of any age, including surgery, chemotherapy, and radiotherapy.

Primary Prevention

Epidemiology of HPV Infection

Human papillomavirus is a key causative agent in the development of cervical cancer. Nearly all cases of cervical cancer can be attributed to HPV infection with the prevalence of HPV infection in cervical cancer as high as 99%.

HPV infection is sexually transmitted. Infection is often asymptomatic. Most sexually active men and women will be infected at some point in their lives, and some may be repeatedly infected. The lifetime cumulative risk of HPV infection is greater than 80%. The peak time for acquiring the infection is shortly after becoming sexually active. Penetrative sex is not essential for transmission, and skin-to-skin genital contact is well-recognized as a mode of infection [3].

Most genital HPV infections are transient and will spontaneously resolve within about 8 months of acquisition, especially in women under the age of 30 years. Viral load is usually undetectable by 2 years in 90% of women. Persistent infection is reported to occur in less than 10% and is defined as the presence of high-risk HPV for longer than 2 years. Persistent infection with HPV increases the risk of cervical cancer [4].

In a national study from the USA, 25% of women between the ages of 14 and 19 years and 45% of women between the ages of 20 and 24 years were HPV positive [5]. The prevalence of HPV in postmenopausal women ranges from 14% to 38%. HPV infection is more likely to persist in women over the age of 65 years, and a positive HPV test is therefore more likely to be clinically significant in this age group [4].

An increasing body of evidence has linked HPV with cancers of the anus, vulva, vagina, and penis. Although these cancers are less frequent than cervical cancer, their association with HPV makes them potentially preventable using similar prevention strategies to those used for cervical cancer.

HPV Subtypes

Human papillomavirus includes over 130 different genotypes that can be subdivided into mucosal and cutaneous. Approximately, 30–40 HPV subtypes infect the genital mucosa and are categorized as low or high risk according to their clinical sequelae: low-risk types primarily cause benign anogenital warts, whereas high-risk types are associated with anogenital cancers.

The proportion of HPV infections that are high risk versus low risk varies with age. For example, adolescents may be at similar risk for low- and high-risk

infections, whereas in women over the age of 30 years, 50–80% of HPV infections are with high-risk subtypes.

HPV-16 and HPV-18 cause approximately 70% of all invasive cervical cancers [4]. HPV types 16, 18, 45, 31, 33, 35, 52, and 58 together cause approximately 95% of cervical cancers [5]. Almost all types of cervical cancer including squamous cancer, adenosquamous cancer, and adenocarcinoma are now thought to be associated with HPV infections. HPV-16 and HPV-18 cause approximately 50% of cervical cancer precursor lesions.

Role of HPV in Malignant Transformation

HPV infects the basal cells of squamous epithelium where keratinocytes undergo differentiation. In most cases the viral DNA stays separate from host DNA and forms an episome. In a subgroup of HPV infections, the viral DNA integrates into host DNA, leading to malignant transformation. The viral E6 and E7 genes inhibit expression of host p53 and retinoblastoma tumor suppressor proteins. These proteins have an important role in cell-cycle control and apoptosis, and inactivation of their genes can induce malignant transformation.

Immunosuppression encourages persistent HPV infection. HIV coinfection can also promote HPV-related malignant transformation at molecular level. Oral contraceptives and hormone replacement therapy (HRT) may upregulate HPV expression. Other risk factors that may play a role in persistent HPV infection include active and passive smoking, host factors such as age and genetics, and external factors, such as nutrition and environment.

HPV Vaccination for the Prevention of Cervical Intraepithelial Neoplasia

Prophylactic vaccines against HPV entered national immunization programs in many countries, including the UK. The development of national vaccination programs against high-risk HPV is one of the most significant recent developments in cervical cancer prevention.

Vaccination generates HPV-specific antibodies that bind to the virus and prevent cervical infection.

There are three types of HPV vaccines currently in use. Cervarix^{R} is a bivalent vaccine that protects against HPV types 16 and 18 (associated with cervical cancer). It is manufactured by GlaxoSmithKline.

Gardasil® (also marketed as Silgard) is a quadrivalent vaccine manufactured by Merck. It protects against HPV types 6 and 11 (associated with anogenital warts) as well as types 16 and 18.

Gardasil 9 is a nine-valent vaccine against HPV types 6, 11, 16, and 18 and types 31, 33, 45, 52, and 58 (responsible for approximately 14% of HPV-associated cancers in women). It is also manufactured by Merck.

The US Food and Drug Administration (FDA) approved the use of Gardasil in females aged 9–26 years in 2006 and use of Cervarix in 2009. The commercialization of Cervarix and Gardasil represents a major milestone in the prevention of cervical cancer. In October 2018 the FDA approved the use of HPV vaccination in females up to an age of 45 years.

The vaccines contain human papillomavirus major capsid protein L1 produced by recombinant techniques. L1 protein assembles into virus-like particles that are identical to HPV virions morphologically but have no viral DNA core. Thus vaccines induce a virus-neutralizing antibody response but pose no infection or oncogenic risk.

In contrast to natural infection, vaccination against HPV is highly immunogenic. It generates high concentrations of neutralizing antibodies to L1, and the virus is neutralized by serum IgG that transudates from capillaries to genital mucosa epithelium.

Gardasil has been approved for vaccination programs in the USA, the UK, Canada, Australia, New Zealand, Spain, France, Switzerland, and Sweden.

In the UK, a nationwide immunization program against HPV infection commenced in September 2008. All girls aged 12–13 years are offered Gardasil at school as part of the NHS childhood immunization program. Girls who have missed early immunization can be offered catchup vaccination until they are 18 years old. For women older than 18 years, HPV vaccination is not covered by the national UK program [6].

The vaccine was initially given as three doses (at 0, 2, and 6 months). Recent randomized controlled trials, however, show similar effectiveness when given in two doses in the 9–14-year-old age group (0 and 6 months) because young adolescents mount a higher immune response. From the age of 15 years, patients should receive the three-dose schedule. The WHO endorsed the use of two-dose HPV vaccination schedules in under 15 years, in 2014. The vaccine is administered as an intramuscular injection.

Very high levels of uptake have been reported in the UK, with 86% and 83% of eligible girls receiving the first and second doses of the HPV vaccine, respectively. This high level of uptake results from the fact that the vaccine is delivered through schools [6].

The USA, Canada, and Australia also offer vaccination to adolescent boys to prevent against anal and oropharyngeal cancers. HPV vaccination is not currently offered to boys or men in the UK. In July 2018, following advice from the Joint Committee on Vaccination and Immunisation (JCVI), the UK Government announced that boys will be included in the HPV schools' vaccination program.

Comparing HPV immunization programs between countries is difficult because the delivery systems often differ significantly [7]. The UK is the only European country that has a national school-based program. Like the UK, Canada and Australia's programs are school based although different provinces target different cohorts of girls between the ages of 9 and 17 years. School-based vaccination programs generally achieve higher coverage than on-demand systems where it relies on

the patient to request the vaccine. Some countries offer vaccination up to the age of 26 years. The effects of vaccination in women of older age have not been sufficiently studied.

Some question the rationale of HPV vaccination in countries like the UK that have well-run screening programs. HPV vaccination will be most beneficial in resource-poor countries that lack organized screening programs and where the burden of cervical cancer is highest.

Evidence of Efficacy

Large international randomized trials have evaluated both Cervarix and Gardasil and have shown that both vaccines are over 99% effective in preventing precancerous lesions associated with HPV-16 and 18 in young women who are HPV naïve [8–10].

Gardasil is also 96–100% effective in preventing anogenital warts (CDC 2015). Gardasil 9 has also been shown to be effective in an international randomized trial [11].

Vaccination results in relatively high antibody titers. A long-term follow-up study of the bivalent Cervarix showed a 95.6% efficacy against HPV types 16 and 18 after 9.4 years. In the case of Gardasil, no precancerous lesions or genital warts related to HPV-6, 11, 16, or 18 were detected at 5-year follow-up [12]. Longer-term studies are however needed to ascertain if booster doses are required.

The major trials have used antibody titers or prevention of CIN2 and CIN3 as primary end points which are surrogate markers for the protection against cervical cancer. Evidence on prevention of cervical cancer is not available [6].

Current vaccines may offer some degree of cross protection against other HPV types; however this effect is probably modest.

There are still unanswered questions about the long-term cost-effectiveness and safety of current HPV vaccines. In the UK there has also been criticism of the decision to limit vaccination to adolescent girls [6].

Safety

In general all three HPV vaccines appear to be safe and well tolerated. They pose no infection or oncogenic risk. The World Health Organization Global Advisory Committee for Vaccine Safety (GACVS) concluded that potential benefits outweigh any harms.

Commonly reported adverse effects include pain, swelling, and redness at the injection site, nausea, headache, fever, musculoskeletal pain, and syncope. Cervarix is recognized to be a more painful vaccination. The anaphylaxis rate for Gardasil has been reported as 2.6 per 100,000 doses which is higher than for other vaccines albeit still rare. An anaphylactic reaction is a contraindication for a subsequent dose.

In the UK, a report of suspected cases of complex regional pain syndrome after HPV vaccination led to a safety assessment report by the Medicines and Healthcare products Regulatory Agency (MHRA), in December 2012. The report concluded that there was insufficient evidence of a causal link with the HPV vaccine [6]. There have been no deaths attributable to the HPV vaccines up to date.

The HPV vaccine is not recommended for use in pregnancy. Where it has been inadvertently administered during pregnancy, no adverse pregnancy outcomes of fetal malformations have been reported [13].

The Future of HPV Vaccination

HPV vaccination does not eliminate the need for regular cervical screening as up to 30% of cervical cancers are caused by HPV subtypes not covered by the vaccine.

While HPV vaccines are unlikely to eliminate the need for effective cervical screening and treatment for many years to come, they can reduce the burden of cervical cancer on women and health services. Significant challenges remain in achieving a greater coverage of adolescents and in reducing the cost of HPV vaccines which can make them more accessible to countries in the developing world, where incidence and mortality is highest [14].

Policy makers debating the use of HPV vaccines in any country should consider the country's disease burden, its health infrastructure and ability to initiate and sustain an immunization program, cultural acceptability, political will, and the cost-effectiveness relative to other programs competing for funding [15].

Great progress is being made to develop novel therapeutic HPV vaccines to treat existing HPV infections and diseases. Therapeutic vaccines aim to generate cell-mediated immunity and may provide a promising nonsurgical option for treating HPV-associated disease [16, 17].

Secondary Prevention

Cervical cancer screening is a way to detect abnormal cervical cells, including precancerous cervical lesions and early cervical cancers. Both precancerous lesions and early cervical cancer can be treated very successfully.

Precancerous cervical lesions better known as cervical intraepithelial neoplasia (CIN) are graded into CIN1, 2, and 3. CIN1 carries a low risk of malignancy. CIN2 and 3, also known as high-grade CIN, carry a risk of progression to cervical cancer if undetected or left untreated.

Cervical cancer screening includes different types of screening tests: cytology-based screening, also known as the Pap test or Pap smear, HPV testing, and visual inspection with acetic acid (VIA).

Cytology-Based Screening

The most widely used cervical screening test is still the Pap smear. A brush is used to sample cells from the cervix under direct vision. Cytology samples are then examined under the microscope to screen for the presence of abnormal cells. Liquid-based cytology has largely replaced conventional Pap tests.

In developed countries with good health resources, Pap smears are the simplest and most accurate method of screening for cervical cancer [18]. The sensitivity of Pap testing has been reported as 78% with a specificity of 62% [4].

It is estimated that the introduction of cervical cytology screening has reduced the incidence of cervical cancer by up to 80% and is particularly effective in preventing advanced disease, thus saving hundreds of thousands of lives [19].

Cervical screening coverage rates vary across western countries and have declined over the past 8 years, particularly in young women. In the UK with its well-founded national screening program, coverage rates have been around 80%. In Italy coverage rates have never been above 50% in young women [20].

Cervical cytology screening programs are resource heavy, and thus uptake is even lower in median- to low-income countries that need them the most. A study investigating the uptake of cervical cytology screening across the globe showed huge variation. They examined 57 countries, and Pap smear uptake averaged 45% in developed and 19% in developing countries with uptake as high as 80% in Luxemburg and as low 1% in Bangladesh [21].

In addition the sensitivity of cytology varies between countries depending on the laboratories and medical infrastructure and ranges from 55% to 94% [4].

Visual Inspection with Acetic Acid

Visual inspection with acetic acid (VIA) or Lugol's iodine (VILI) is a simple and cheap method used in many screening programs in low-income countries. The cervix and the squamocolumnar junction are visualized with a colposcope, and a small amount of 3–5% acetic acid (or Lugol's iodine) is applied directly onto the cervix. Areas with a high mitotic activity will temporarily appear white (or iodine negative) potentially indicating the presence of cervical intraepithelial neoplasia (CIN). Tumors or similar gross abnormalities can be classified as suspected cancer. With a VIA-positive result, the abnormal area can be either biopsied or excised. The absence of white or iodine-negative area constitutes a VIA-negative result.

VIA can be performed on opportunistic basis at any time during the menstrual cycle, immediately postpartum, or after pregnancy termination without need for an expensive laboratory setup. There is less reliance on sequential testing, therefore empowering the examining clinician "to see and treat" any lesions or area of abnormality.

VIA has a specificity of 82% and sensitivity of 84% owing to a high false-positive rate [4]. Diagnoses are not confirmed until histology results are available. There is a risk therefore of overtreating or excising benign tissue or CIN1, particularly in

areas with high HIV prevalence where women are more likely to have positive results. In addition the transformation zone where most of the abnormalities are found is harder to visualize in postmenopausal women [22].

HPV-Based Screening

The development of molecular testing for HPV has opened up the potential for self-testing, especially in low-resource countries. HPV self-testing can overcome the potential obstacles of access to trained healthcare professionals as well as cultural variations in attitude toward intimate examinations.

Self-sampling for HPV detection shows high concordance (96.8%) with physician taking sampling [23].

HPV testing alone appears to be more sensitive compared with cytology. The sensitivity of HPV testing for the detection of CIN2 and CIN3 has been reported as 94.6% compared to 55.4% for Pap testing [24]. Its negative predictive value is high.

It is, however, less specific and has a lower positive predictive value compared with cytology. Despite a lower positive predictive value, HPV testing may be preferred in low-resource countries where restricted infrastructure reduces the effectiveness of cytology screening programs. In addition, because women in low-resource settings will be screened only a few times in their lives, the high sensitivity of HPV testing is of paramount importance [25].

Other biomarkers may be used after HPV testing in the future to improve the specificity of screening. Developing such biomarkers would reduce the number of women being unnecessarily referred to secondary care for colposcopy [20].

WHO Guidance

Prior to 2013, the WHO advised regular cervical cytology testing every 3–5 years for all women aged 25–49 years and then 5 yearly until the age of 65. If abnormal cells were detected, women should be invited for colposcopy screening. For CIN2 and CIN3 lesions, the patient should have a biopsy or undergo excision of the abnormal area with large loop excision of the transformation zone (LLETZ) or cold knife conization, serving as both a biopsy and treatment [26].

In 2013 the WHO published new guidance on cervical screening, incorporating HPV testing in women over the age of 30 to help address the lack of established screening programs in low-resource countries as well as support existing cytology-based screening. They recommend a “see and treat” approach aiming to reduce the number of steps between screening and treatment [27].

In countries with established and successful cervical cytology screening programs, the WHO advised adding HPV testing in colposcopy follow-up. In countries with VIA screening only, the WHO advised introducing HPV testing as a first screening test and using VIA as second testing and method to direct treatment. A negative HPV test can extend the screening interval to 5 years.

The Future of Cervical Screening

The cohorts of women who were offered HPV vaccination in 2007/2008 will reach the age of first cervical screening (25 years) from 2017. As HPV-related precancerous lesions become increasingly rare in countries where HPV vaccines are available, there may be a need for major reorganization of cervical cancer prevention.

An important objective is to define evidence-based screening strategies for girls vaccinated against HPV. Tailored screening protocols based on vaccination status, rather than "a one-size-fits-all approach," are needed until a herd immunity effect is achieved.

A consensus conference that took place in Italy in 2015 recommended that vaccinated women should start screening at the age of 30 years with HPV test. There is a strong rationale for applying longer intervals for rescreening HPV-negative women although more research is needed to define an optimal interval. Longer screening intervals and delayed onset of screening can have a positive economic and organizational impact by reducing workload and unnecessary referrals and treatments. For non-vaccinated women and women vaccinated at the age of 15 years, the Italian consensus conference recommended the following current protocols [28]. The UK will introduce HPV as primary screening in 2019.

Tertiary Prevention: Treatment

Even in countries with good screening and prevention strategies, women will still succumb to cervical cancer. For example, in England and Wales where a national call/recall program for cervical screening was established in 1988 [29], three women a day still die of cervical cancer [30]. The burden of cervical cancer is significantly higher in developing countries where screening may be ad hoc or indeed unavailable. The WHO defines tertiary prevention of cervical cancer as access to cancer treatment and management for women of any age, including surgery, chemotherapy, and radiotherapy. When curative treatment is no longer an option, access to palliative care is crucial [31].

Clinical Presentation

Cervical cancer can present in a variety of ways. Screen-detected cancers are diagnosed when a woman attends for cervical screening. The screening report may raise the possibility of an invasive lesion in an asymptomatic woman with a macroscopically normal cervix, prompting further investigation. These tumors are small and curable by local measures in the vast majority of cases. Sometimes there is a visible lesion on the cervix on inspection. Direct questioning in such cases may reveal symptoms, the significance of which the patient may not have recognized.

Table 2.1 Symptoms of advanced cervical cancer

	Symptoms
Advanced cervical cancer	Urinary frequency and urgency Low abdominal pain Backache Weight loss Anuria (secondary to obstructive renal failure) Vaginal urinary or fecal loss (secondary to fistulation) Lower limb edema Cough, shortness of breath (pulmonary edema, effusions, or metastasis)

Pecorelli [33]

The common presenting symptoms of cervical cancer are typically postcoital, intermenstrual, or postmenopausal bleeding and an offensive vaginal discharge. Women may present with symptoms of metastasis in more advanced disease (Table 2.1).

Clinical Assessment

Any woman with symptoms suggestive of cervical cancer should have a full clinical examination, which includes visualization of the cervix, using a speculum. Cervical screening is not indicated if there is a suspicion of cancer. Screening tests are designed for an asymptomatic population. There may be an exception however. Lim et al. [32] have suggested that a smear may assist primary healthcare providers in diagnosing cervical cancer more effectively.

Women with suspected cervical cancer should be assessed by a specialist. In women who present with an abnormal smear, colposcopy may be indicated, with biopsy of the abnormal area. If the lesion is small, an excision biopsy with cold knife or laser may be performed with curative intent.

Staging of Cervical Cancer

Treatment of cervical cancer depends on the FIGO stage (Table 2.2) at which it is diagnosed, so every attempt should be made to stage the disease accurately. Staging is by clinical assessment, but this may be aided by cross-sectional imaging where facilities are available.

Initial assessment should include a full-blood count to exclude anemia, renal, and liver function tests. Additional blood tests may be considered if bony metastases are suspected.

Staging is traditionally performed by examination under anesthesia with cystoscopy and sigmoidoscopy if indicated. Any abnormal tissue is biopsied for histopathological diagnosis. An MRI scan will enable more exact assessment of parametrial involvement. Assessment of ureteric involvement or renal function can be assessed by urogram or CT of the chest abdomen and pelvis which has the additional advantage of excluding nodal and other metastatic disease.

Table 2.2 Revised FIGO staging of cervical carcinoma 2009

Stage 0: cervical intraepithelial neoplasia (HSIL or CIN III)
Stage I: confined to cervix
Stage Ia: invasive carcinoma only diagnosed by microscopy
Stage Ia1: stromal invasion <3 mm in depth and < 7 mm in extension (*microinvasive*)
Stage Ia2: stromal invasion >3 mm depth and not >5 mm and extension <7 mm
Stage Ib: clinically visible lesions limited to the cervix or preclinical cancers >stage 1a
Stage Ib1: clinically visible tumor <4 cm in greatest dimension
Stage Ib2: clinically visible tumor >4 cm in greatest dimension
Stage II: beyond cervix though not to the pelvic sidewall or lower third of the vagina
Stage IIa: involves upper 2nd/third of the vagina without parametrial invasion
Stage IIa1: clinically visible tumor <4 cm in greatest dimension
Stage IIa2: clinically visible tumor >4 cm in greatest dimension
Stage IIb: with parametrial invasion
Stage III
Stage IIIa: tumor involves the lower third of the vagina with no extension to pelvic sidewall
Stage IIIb: extension to pelvic side wall or causing obstructive uropathy; MR imaging findings that are suggestive of pelvic sidewall involvement include tumor within 3 mm of or abutment of the internal obturator, levator ani, and pyriform muscles and the iliac vessel
Stage IV: extension beyond true pelvis or biopsy proven to involve the mucosa of the bladder or the rectum
Stage IVa: extension beyond true pelvis or rectal/bladder invasion
Stage IVb: distant organ spread

From Pecorelli [33], with permission

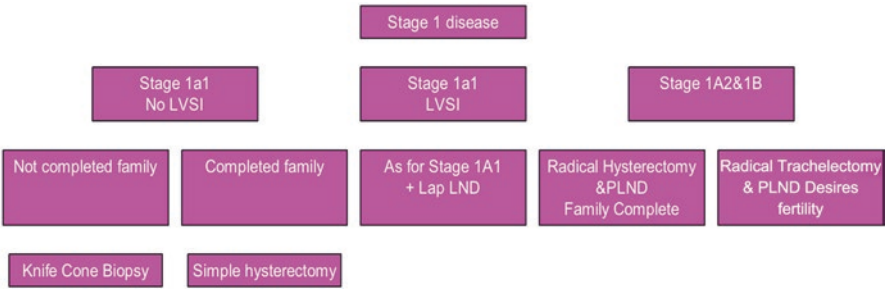

Fig. 2.1 Surgical management of cervical cancer

A test for infection with the human immunodeficiency virus (HIV) should be part of the assessment protocol.

Treatment

Women presenting with FIGO stage I cancer can be managed surgically. Local excision is sufficient for stage 1Ai lesions in women desirous of fertility. More radical treatment is required for larger tumors (Fig. 2.1). Where hysterectomy, simple or

radical, is required, the laparoscopic approach is associated with less morbidity and should be offered where the expertise is available. Larger tumors or those with poor prognostic factors are treated with chemoradiation.

References

1. Bray F, et al. Global estimates of cancer prevalence for 27 sites in the adult population in 2008. Int J Cancer. 2013;132(5):1133–45.
2. Ferlay J, et al. GLOBOCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr
3. Human papilloma virus (HPV) and cervical cancer WHO Fact Sheet, Updated June 2016 http://www.who.int/mediacentre/factsheets/fs380/en/
4. Goodman A. HPV testing as a screen for cervical cancer. BMJ. 2015;350:h2372.
5. Kahn J. HPV vaccination for the prevention of cervical intraepithelial neoplasia. N Engl J Med. 2009;361(3):271–8.
6. Quah YL, Aggarwal IM. 10 minute consultation. Discussing human papilloma virus vaccination. BMJ. 2017;357:j2730.
7. Crosbie EJ, Brabin L. Cervical cancer: problem solved? Vaccinating girls against human papillomavirus. BJOG. 2010;117(2):137–42.
8. Garland SM, et al. Females United to Unilaterally Reduce Endo/Ectocervical Disease (FUTURE) Investigators. Quadrivalent vaccine against human papilloma virus to prevent anogenital diseases. N Engl J Med. 2007;357:1928–43.
9. FUTURE II Study Group. Quadrivalent vaccine against human papilloma virus to prevent high-grade cervical lesions. N Engl J Med. 2007;357:1915–27.
10. Centers for Disease Control and Prevention. Human papilloma virus (HPV) vaccine safety. CDC, 2015. www.cdc.gov/vaccinesafety/vaccines/hpv-vaccine.html
11. Joura EA, et al. A 9-valent vaccine against infection and intraepithelial neoplasia in women. N Engl J Med. 2015;372(8):711–23.
12. De Vincenzo R, et al. Long term efficacy and safety of human papilloma virus vaccination. Int J Womens Health. 2014;357:999–1010.
13. Narducci A, Einarson A, Bozzo P. Human papilloma virus vaccine and pregnancy. Can Fam Physician. 2012;58(3):268–9.
14. Ma B, Roden R, Wu TC. Current status of HPV vaccines. J Formos Med Assoc. 2010;109(7):481–3.
15. Agosti JM, Goldie SJ. Introducing HPV vaccine in developing countries – key challenges and issues. N Engl J Med. 2007;356(19):1908–10.
16. Trimble CL, Frazer IH. Development of therapeutic HPV vaccines. Lancet Oncol. 2009;10(10):975–80.
17. Yang A, et al. Perspectives for therapeutic HPV vaccine development. J Biomed Sci. 2016;23(1):75.
18. Vilos GA. The history of the Papanicolaou smear and the odyssey of George and Andromache Papanicolaou. Obstet Gynecol. 1998;91(3):479–83.
19. Sasieni P, Castanon A, Cuzick J. Effectiveness of cervical screening with age: population based case-control study of prospectively recorded data. BMJ. 2009;339:b2968. https://doi.org/10.1136/bmj.B2968.
20. Martin-Hirsch P. Commentary on 'Use of a high-risk human papilloma virus DNA test as the primary test in a cervical cancer screening programme: a population-based cohort study'. BJOG. 2013;120(10):1267–8.
21. Gakidou E, Nordhagen S, Obermeyer Z. Coverage of cervical screening in 57 countries: low average levels and large inequalities. PLoS Med. 2008;5(6):e132. https://doi.org/10.1371/journal.pmed.0050132.

22. World Health Organisation International Agency for Research on Cancer Screening Group. Visual Inspection with Acetic Acid: Evidence to Date. 2002. http://screening.iarc.fr/doc/RH_via_evidence.pdf. Accessed 11 June 2017.
23. Ketelaars PJW, et al. High risk human papilloma virus detection in self-sampling compared to physician taken smear in a responder population of the Dutch cervical screening: Results of the VERA study. Prev Med. 2017;101:96–101.
24. Mayrand MH, et al. Human papilloma virus DNA versus Papanicolaou screening tests for cervical cancer. N Engl J Med. 2007;357(16):1579–88.
25. Lazcano Ponce E, et al. Self-collection of vaginal specimens for human papilloma virus testing in cervical cancer prevention (MARCH): a community-based randomized controlled trial. Lancet. 2011;378(9806):1868–73.
26. World Health Organisation, Department of Reproductive Health and Research and Department of Chronic Diseases and Health Promotion, Geneva. *Comprehensive cervical cancer control: a guide to essential practice* (C4-GEP) 2006. http://www.who.int/reproductivehealth/publications/cancers/9241547006/en. Accessed 11 June 2017.
27. World Health Organisation. WHO Guidelines for Screening and Treatment of Precancerous Lesions for Cervical Cancer Prevention. © World Health Organization 2013 ISBN 978 9241548694.
28. Rossi G, et al. Cervical cancer screening in women vaccinated against human papilloma virus infection: recommendations from a consensus conference. Prev Med. 2017;98:21–30.
29. Cervical Cancer Incidence and Screening Coverage, NCRAS. http://www.ncin.org.uk/publications/data_briefings/cervical_incidence_and_screening
30. Cervical Cancer Mortality Statistics, Cancer Research UK. http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/cervical-cancer/mortality
31. World Health Organization: Global Atlas of Palliative Care at the End of Life, 2014. www.who.int/nmh/GlobalAtlasofPalliativeCare
32. Lim AW, et al. Cytology in the diagnosis of cervical cancer in symptomatic young women: a retrospective review. Br J Gen Pract. 2016;66(653):e871–9. Epub 2016 Oct 24
33. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix and endometrium. Int J Gynaecol Obstet. 2009;105(2):103–4.

Current Advances in Optical Screening for Cervical Cancer

3

Amuthachelvi Daniel and Wilfred Prasanna Savarimuthu

One of the greatest economic burdens faced by the world today is health care. One significant way to alleviate this burden is by reducing morbidity and mortality. This can be achieved by increasing the competence of a system to diagnose the early onset of a disease, in real time, objectively, noninvasively and unambiguously. This also holds the key for better therapeutic prognosis. The currently available clinical tests generally rely on single disease markers and are subjective. This makes these tests unreliable, and a number of repeat tests and follow-up examinations are required, increasing the anxiety of the patient and the economic burden. This lacuna can be addressed by molecular-level diagnosis. Hence the research community is in pursuit of molecular fingerprinting which differentiates samples between different biological conditions with emphasis on real-time, high-throughput analysis.

Light Interaction with Tissue

Light is partially reflected or scattered at the surface of the tissue, part of it is absorbed, some of the absorbed is emitted, and part is transmitted. The scattering process takes place since different cellular structures have different refractive indices. The reflection depends on the angle of incidence. As the angle of incidence decreases, reflection of light decreases. Therefore when the incident light is perpendicular to the sample, the least reflection occurs. Some biomolecules like haemoglobin, melanin and water, known as chromophores, are present in the tissues which absorb the photons. A small fraction of the incident light is transmitted, and with increased wavelength, the transmitted depth increases.

A. Daniel (✉)
Department of Medical Physics, Anna University, Chennai, Tamil Nadu, India

W. P. Savarimuthu
Department of Physics, Madras Christian College, Chennai, Tamil Nadu, India

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_3

Diffuse Reflectance Spectroscopy

Diffuse reflectance spectroscopy (DRS) is a reliable, quantitative tissue characterization tool, where the principle involved is through reflection at altered angle, a phenomenon due to the tissue surface roughness. It is even correlated with inelastic scattering, since it is basically evaluating the spectral signature of backscattered light [1]. DRS coupled with other technologies like optical fibre probe, staining or applying acetic acid elevates this technique to be noninvasive and sensitive and enables to even monitor precancerous changes of the cervix [2]. Most of the present DRS systems either has a broadband source with a multispectral detector or a fibre to irradiate and acquire the reflected light from the target. The altered reflectance signature of neoplastic tissue is primarily due to the collagen fibres and organelles of epithelial and stroma [3].

Fluorescence Spectroscopy

When the sample is exposed to photons of energy sufficient to cause electronic transition within the molecule, the photons are absorbed resulting in the molecule moving to higher electronic energy level. Usually the absorption occurs at singlet ground state to the first electronic state. With a lifetime of a few microseconds, the molecule moves to lower vibrational levels, and the energy dissipates in the form of heat. Although the energy level is discrete, the obtained spectra have a broad peak indicating that photons from the first excited electronic state return to any of the various vibrational levels within the singlet ground state, emitting fluorescent photons of varying wavelengths (Fig. 3.1).

Fluorescence Lifetime

The average time a molecule is in the excited state before returning to ground state is the fluorescence lifetime of the molecule. This lifetime depends upon the microenvironment of the molecule. Hence this property has been exploited to study the microenvironment of cells and tissues with the aim of diagnosis of disease.

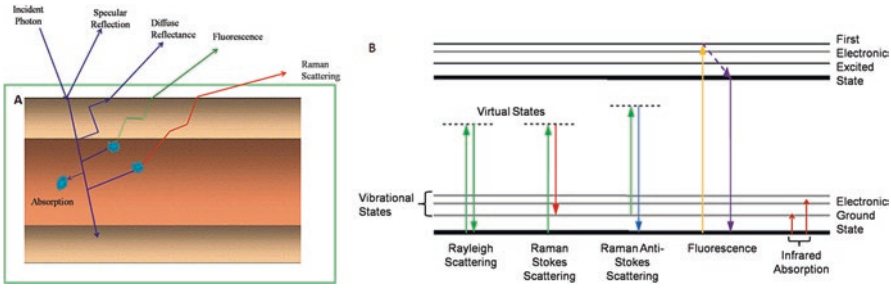

Fig. 3.1 (**a**) Schematic representation of light-tissue interaction. (**b**) Energy-level diagram of Raman scattering, Rayleigh scattering, IR absorption and fluorescence

Raman Spectroscopy

Conventional Raman Spectroscopy

Raman scattering is an inelastic scattering process in which vibrational modes in molecules are excited by the interaction with the incident light. This Raman scattered photon (Fig. 3.1) can either occupy a higher virtual energy state (anti-Stokes) or lower virtual energy state (Stokes). This depends upon the interaction of light with the various vibrational modes associated with chemical bonds of the sample. Hence qualitative chemical information of the compound can be obtained.
Further the low signal of water makes it an excellent candidate for analyses of biological materials.

On the other hand, the probability of Raman scattering is low and is of the order of one Raman photon for every 100 million photons. With the advent of highly efficient laser sources, charge-coupled devices (CCDs), effective filters and optics, this technique, which was regarded as insensitive technique, has gained importance in the last couple of decades. Further in vivo biological investigations are made possible by efficient fibre-optic probes. Furthermore the development of confocal microscope coupled to the Raman spectroscope has greatly extended the horizon of its utility to Raman imaging of biological samples.

Surface-Enhanced Raman Spectroscopy (SERS)

Since Raman effect is a weak phenomenon, researchers around the globe have tried to enhance the weak effect. One such novel technique is enhancing the Raman signals by nanoparticles known as SERS. This technique enhances the Raman signals from Raman active molecules adsorbed onto certain metal surfaces. This is because the surface plasmon resonance is the resonant oscillation of conduction electrons at the interface between a negative and positive permittivity material stimulated by incident light. The signal strength increases of the order of 10^3–10^{10} and has high levels of molecular specificity [4, 5].

Fourier-Transform Infrared (FTIR) Spectroscopy

The photons of infrared radiation do not possess enough energy to excite electrons, but the energy is sufficient to induce bond vibration. Hence these photons are absorbed, and the phenomenon is called as IR absorption. The measurement technique to obtain the spectrum for the various bond vibrations of a given sample is achieved by FTIR spectroscopy. The mathematical Fourier transform of the signal from the interferometer is carried out to obtain the FTIR spectra.

The sample is exposed to infrared radiation of a single wavelength in conventional IR spectroscopy at a time. To obtain the entire spectra, the sample has to be exposed to different wavelengths, a time-consuming process [6], whereas in FTIR spectroscopy, the sample can be exposed to the entire region of wavelengths simultaneously with a single beam.

Attenuated total reflection (ATR) in conjunction with FTIR spectroscopy facilitates the analysis of both solid and liquid samples. The ATR crystal through which the IR radiation is passed through causes total internal reflection. The crystal is inclined at 90° to the sample. Pressure contact is made between the sample and the crystal to eliminate the inaccurate measurement that may be obtained by the air trapped between them [7]. The photons from the evanescent wave are absorbed by the various molecules within the sample; hence the absorbance spectrum is obtained. This technique holds a risk of sample damage due to pressure applied. With the dawn of various optoelectronic components, IR spectroscopy has risen to the challenge and successfully contributed to the molecular-level mapping or imaging of tissues.

Statistical Analysis

The aid of multivariate statistical analysis is inevitable to glean meaningful inferences from the enormous data set obtained from these spectroscopic studies. A heuristic approach has been carried out for the data set utilizing the many multivariate statistical analyses. In a nutshell, the sequence of the optical diagnosis is shown in Fig. 3.2.

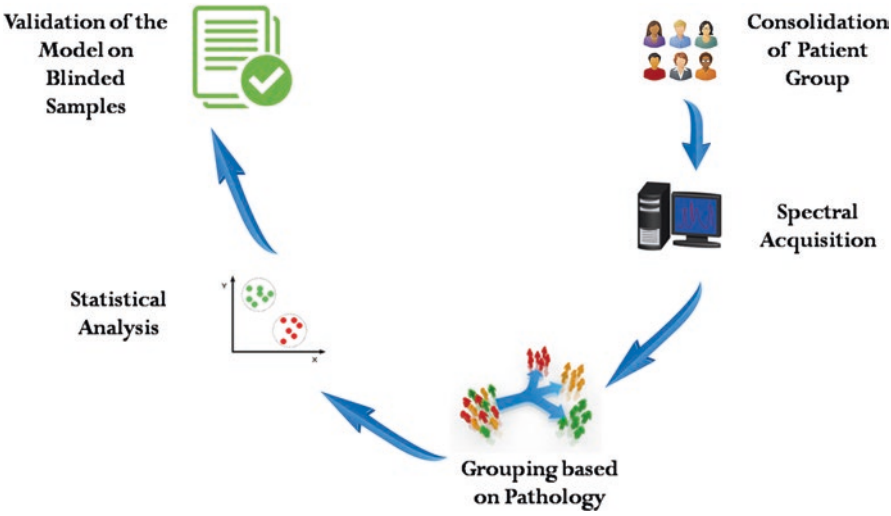

Fig. 3.2 Schematic flow chart of optical diagnosis methodology

Screening Modalities

Current Modalities

The early twentieth century laid the cornerstone for the current screening modality of cervical cancer based on optical techniques. A simple light specially designed microscope called colposcope is used to visualize the cervix, vagina and vulva, after an abnormal Pap smear. The precancerous lesion is characterized by changes in the reflectance property of the tissue. The various other methods employed for simple visual examination include the application of acetic acid and Lugol's iodine coupled to a green filter to highlight the suspicious areas. Nonetheless, the accuracy of diagnosing is poor by these techniques [8]. Therefore a further pathological study is required to establish the stage of the disease. This in turn calls for skilled personal and lab facilities. The incidence of cervical cancer is higher in the underdeveloped and developing countries; women in these parts of the world do not have access to life-saving screening programmes. Therefore, in these countries, a simple, cost-effective, reliable, objective and visual examination would be a boon. Hence researchers have extensively explored for techniques to address to this.

Diffuse Reflectance Spectroscopy

Steady-state reflectance spectroscopy is a technique where light after multiple scattering in the tissue is collected and studied for qualitative and quantitative information. Nordstrom et al. have studied the diagnostic prospective of fluorescence and reflectance spectroscopy of four different types of tissue samples. The average reflectance spectra of individual tissue types show distinguished difference in the spectral signatures. The data exhibit excellent ability to distinguish from the tissue types even as low as the tissue differences between CIN II and CIN III [9]. Further, a study of reflectance spectroscopy involving 324 sites of 164 precancerous patients carried out by Mirabal et al. has found the discrimination ability between squamous normal and high-grade squamous intraepithelial lesions with sensitivity and specificity of 72% and 83% respectively [10]. Further studies have also successfully demonstrated the diagnostic prowess of DRS even at its precancerous stages [11–14].

Although DRS facilitate many advantages, even a slightest error like probe-to-tissue pressure can alter the accuracy of the spectra. Yu et al. have attempted to develop a portable device using optical fibre probe equipped with optical components to reduce such operator errors [2]. Various additional information through Monte Carlo modelling have only advanced the reflectance spectroscopy. For instance, Wang et al. have worked on the layer-specific optical properties coupled with angular variation of fibre geometry attempting to resolve spatially specific spectra in reflectance-mode Monte Carlo simulation [15]. Arifler et al. propose a probe design of source and detector coupled with half-ball lens which is expected to resolve two-layer spectral information of epithelial and stromal scattering [16].

Orfanoudaki et al. [17] have worked with 123 subjects and have comparative results between histopathology, multispectral imaging (MSI) colposcopy, Pap smear and conventional colposcopy. The results suggest MSI colposcopy could offer improved diagnostic information in a narrow spectral range with high spatial resolution and offers enhanced image contrast using simple tools like polarizer [17]. The approach is that the image will be created with series intensity values extracted from the data at various wavelengths [1].

Various studies have investigated MSI coupled with reflectance and have reported green wavelength provides the best imaging contrast due to haemoglobin absorption [18], whereas white illumination and subsequent separation of white light to different colours result in coloured reflectance images [19]. A study on 29 subjects using multispectral digital colposcope to acquire reflectance imaging and comparing with histopathology standard reveals 79% sensitivity and 88% specificity on employing automated image analysis algorithm [20]. Using polarization in such image acquiring is found to greatly enhance the visualization of even the sub-epithelial pattern [18]. Further application of acetic acid is found to significantly increase reflection in terms of intensity as well as persistence for a prolonged time [21].

Confocal microscopy is a technique which provides real-time 2D and 3D images offering insight information of tissue architecture and cellular morphology. For instance, pre- and post-acetic acid-stained confocal 2D and 3D images of nine precancerous samples were studied by Collier et al. In this study a linear discriminant function was employed which was based on the nuclear-to-cytoplasmic ratio and scattering coefficient. This approach provides this study with 100% sensitivity and specificity to the entire data set [22].

Present studies focus on simultaneous multispectral analysis which provides higher sensitive and accurate diagnosis. Alvarez et al. have worked on a detection system which involves fluorescence, white reflectance and video imaging. The results show the diagnostic capability only improves as more techniques are coupled and operated simultaneously [13]. A comparative analysis of DRS and Raman spectroscopy in cervical cancer by R. Shaikh et al. has showed Raman spectroscopy to be more precise in terms of diagnostic competence, but DRS is suitable for mass screening owing to its lower cost and portability [23].

Fluorescence Spectroscopy

Fluorescence spectroscopy and its variants are used in the field of oncology for its diagnostic prowess. This technique is comparatively the most exploited of spectroscopic techniques for cancer diagnosis. The first comprehensive study started as early as 1960 performed by Winkelman and Rasmussen-Taxdal [24], in an attempt to study the fluorescence of porphyrins chemically extracted from the tissue. Subsequent studies to date intend to diagnose premalignancy state or assessing the severity of the malignancy using diverse fluorescence techniques [25]. The diagnostic potential of fluorescence spectroscopy in the cervix over other conventional techniques is prominently highlighted in a review by Mitchell et al. [26]. Fluorescence

diagnosis is based on acquiring emission spectral alterations of selective chromophore due to biochemical changes, followed by statistical analysis using appropriate tool and interpretation with noteworthy parameters. Various chromophores are studied in this regard including nicotinamide adenine dinucleotide (NADH), collagen, elastin, porphyrins and carotenoids [27].

Autofluorescence of chromophores depends on the choice of the excitation wavelength. Cervical epithelial cells emit cytoplasmic autofluorescence of mitochondrial NADH at wavelength ranging approximately 330–370 nm observed prominently in basal epithelial cells, and wavelength ranging from 510 to 550 nm could be used to probe mitochondrial FAD. Cytokeratins in the cervical epithelial cells also fluoresce at the outer layer of the cells upon excitation at UV and visible range. Collagen cross-links in the normal tissue fluoresce at wide range of wavelengths which tends to increase with age and menopause. It is also observed that such fluorescence of collagen cross-links at the stroma reduces in case of cervical precancers and cancers [21, 28]. A prominent cytoplasmic fluorescence is observed in the normal epithelium along with peripheral fluorescence by superficial cells. However, the cytoplasmic fluorescence dominates the peripheral fluorescence on neoplastic process [1, 29]. A list of endogenous fluorophores with excitation and emission maxima was listed in a review by Nirmala Ramanujam [30].

Steady-state fluorescence spectra at 355 nm excitation were performed on 78 cases and consecutively approximately with several Gaussian components. The results conclude that the spectral regions 402–416 nm and 424–438 nm are crucial for the discrimination of normal and CIN2+ groups and the spectral regions 480–515 nm and 595–625 nm are significant for the identification of cervicitis [31]. Further, Pandey et al. have attempted to study the collagen and NADH fluorescence changes in a study sample of 46 patients in vivo using an optical fibre probe. This study reveals that high false-negative results limit conventional Pap smear test and false-positive results limit colposcopy. Further, the result shows that on considering stromal and epithelial fluorescence together, the accuracy level is 96.5%, and the false negative is merely 4.34%. However, there is absence of false-positive findings in this study due to lack of control [32].

Ramanujam et al. have attempted to study the in vivo autofluorescence of cervix at colposcopy. They include a study population of 28 patients on 66 colposcopically normal areas and 49 histopathologically abnormal areas including pathological inflammation, HPV infection and different grades of cervical intraepithelial neoplasia (CIN). It is shown that CIN could be diagnosed with a sensitivity and specificity value of 87% and 73%, with a positive predictive value of 74% [33]. This study showed fluorescence spectroscopy could be positively used for detection of viruses like HPV [27]. Further the same group went on to develop multivariate statistical algorithm, and this study showed specificity could significantly improve relative to colposcopy [34].

In a 34-patient study, Drezek et al. have engaged fresh colposcopically prepared normal and dysplastic biopsied tissue section and analysed the autofluorescence at 380 nm and 460 nm. The 380 nm excitation induces increase in fluorescence intensity in epithelial layer which is attributed to NADH and stromal layer. They have

also observed a decrease of 17–40% in the redox ratio of dysplastic tissue which points out the increase in the metabolic activity. The bright-field images of this study reveal the comparative increase of fluorescence intensity of the dysplastic tissue. They have also speculated the accuracy in the fluorescence emission of the frozen-thawed tissue and have reasoned out the redox state could be altered causing NADH to be oxidized to NAD+ (nonfluorescent), during the sample preparation of the frozen tissue [29]**.** Along with NADH and FAD fluorescence, Mujat et al. have studied the autofluorescence of HPV-immortalized cells; the tryptophan fluorescence images show intensity as well as localization differences between normal and HPV-immortalized cells [35].

Topical application of markers is also attempted to enhance the accuracy of fluorescence diagnosis. Vansevičiūtė et al. have used 3% of 5-ALA cream to be applied topically, and the spectral signature at 634 nm for PPIX and autofluorescence intensity at 510 nm were evaluated. The results show fluorescence sensitivity is of 91.2% for each patient's data which was higher than colposcopy value of 88.2% [36]. Differentially expressed proteins of the cervix were analysed by Zhao et al. using two-dimensional fluorescence difference in gel electrophoresis and DeCyder software which were used to detect the differentially expressed proteins. The study concludes stating that the differently expressed proteins could be a potential candidate as a marker in early cervical cancer diagnosis and provides helpful information on the developing of CIN to cervical squamous cell carcinoma [37].

Raman Spectroscopy

In Vitro Studies

The year 1991 marked the pioneering work of Alfano. He had acquired Raman spectra for the tissues from the gynaecological tract (i.e. cervix, endometrium, ovarian tissue) using FT-Raman spectroscopy. In that study the group had employed the relative intensities of relevant peaks to distinguish between normal, benign and cancerous tissues. Mahadevan-Jansen et al. [38] acquired Raman spectra of 36 cervical tissue biopsies from 18 patients using excitation of 789 nm laser [38]. This study distinguished precancer from benign tissue with a sensitivity of 82% and a specificity of 92% using principal component analysis (PCA) combined with Fisher discriminant analysis.

The formalin-fixed paraffin-preserved histological samples of normal, CIN and invasive cervical carcinoma tissues from 40 patients were subjected to Raman spectroscopic analysis by Lyng et al. [39]. In order to gain an insight into the molecular-level changes due to oncogenesis, Raman spectra from pure biochemicals such as proteins, nucleic acids, lipids and carbohydrates were obtained. The study discriminated the pathologies with promising results with sensitivity of 99.5%, 99% and 98.5% for normal, CIN and invasive carcinoma, respectively, whereas the specificity was 100%, 99.2% and 99% for normal, CIN and invasive carcinoma, respectively.

Jess et al. have explored the spectral characteristics of the various defined cell lines infected with human papilloma virus (HPV 16) expressing E7 gene. The study concluded that the technique was used accurately and objectively proving the concept that Raman spectroscopy is indeed a valuable approach for the identification and discrimination of different stages of HPV-associated neoplasia [40].

Krishna et al. showed the various spectral features characterizing the normal and malignant tissue samples. In order to develop highly objective discrimination methods, very elaborate data analysis was carried out using PCA. Standard sets for normal and malignant were prepared and tested retrospectively and prospectively. Several parameters such as scores of factor, Mahalanobis distance and spectral residuals were explored for discrimination, and very clean clustering of normal and malignant spectra was achieved in the tissues. This analysis has produced very high, 99.5%, sensitivity and specificity [41].

In Vivo Studies

The promising results from in vitro studies had encouraged the researchers across the world to explore in vivo Raman spectroscopy for clinical cancer management and screening of early cancer. The development of a compact fibre-optic probe by Anita Mahadevan-Jansen and colleagues has paved way for a new line of approach for cervical cancer and precancer management by means of Raman spectroscopy during clinical colposcopy [42]. They had reported that above 900 cm^{-1}, the in vivo spectra resembled the in vitro spectra except for three exceptions. The other major conclusion drawn from this study was the reduction of integration time from 90 s to 20 s could be achieved by increasing the power of laser source. Further the same group [43] had reported an in vivo study for 13 patients recruited during colposcopy. The in vivo Raman spectra were measured from normal, inflammation, squamous metaplasia and low-grade and high-grade precancer cervical tissue sites and successfully differentiated the high-grade precancer using intensity ratio algorithms.

Amy Robichaux-Viehoever et al. have reported a substantial study of 79 subjects with a clinically feasible 5 second integration time [44]. They had employed logistic regression discrimination algorithms for the classification of samples as normal ectocervix, squamous metaplasia and high-grade dysplasia. Independent sets of data were utilized for training and validation. The study has achieved a sensitivity of 89% and specificity of 81% for discriminating high-grade dysplasia and benign tissue.

Mo et al. had reported for the first time a study by employing high-wavenumber Raman spectroscopy [45]. They had recruited 46 subjects and acquired 92 spectra. This work showed the major differences in Raman intensities of the prominent bands are 2850 and 2885 cm^{-1} (CH_2 stretching of lipids) and 2940 cm^{-1} (CH_3 stretching of proteins), and the broad Raman band of water (peaking at 3400 cm^{-1} in the 3100–3700 cm^{-1} range) was observed in normal and dysplasia

cervical tissue. The statistical analysis yielded a sensitivity of 93.5% and specificity of 97.8% for the discrimination of dysplasia.

Kanter et al. studied the discrimination samples based on fingerprint region of Raman spectroscopy [46, 47]. They had employed a novel statistical tool, namely, maximum representation and discrimination feature (MRDF) to extract diagnostic information with sparse multinomial logistic regression (SMLR) to classify the spectra. The results obtained had significantly better classification accuracy resulting in 98% sensitivity and 96% specificity. The same group had further included the hormonal status to increase the accuracy of discriminating low-grade squamous intraepithelial lesion (LGSIL) with an accuracy of 97% [48].

Duraipandian et al. had employed genetic algorithm-partial least squares-discriminant analysis (GA-PLS-DA) to achieve better accuracy. They had recruited 29 patients and obtained 105 spectra. The accuracy by this technique was 82.9% [49]. The same group has also reported the simultaneous use of both fingerprint and high-wavenumber spectra for classification [50]. The statistical technique employed was multivariate diagnostic algorithms based on partial least squares-discriminant analysis (PLS-DA). The overall diagnostic accuracies are 80.3%, 74.2% and 82.6%, using fingerprint, high-wavenumber and integrated fingerprint and high-wavenumber Raman spectroscopic techniques, respectively.

Liquid Biopsy

A pilot study has been reported by José Luis González-Solís et al. on serum obtained from 19 patients who were clinically diagnosed with cervical cancer, 3 precancer and 20 healthy volunteer controls [51]. One hundred fifty spectra were totally obtained from cervical cancer and precancer serum samples and 138 spectra from normal subjects. These spectra were dimensionally reduced by principal component analysis; thereof the scores were used for discrimination. The sensitivity thus obtained was 100% and specificity was 97.1%.

Surface-Enhanced Raman Spectroscopy (SERS)

Shangyuan Feng et al. have explored the feasibility of SERS for liquid biopsy [52]. In this work, they had reported the analysis of blood plasma with silver nanoparticles and the statistical analysis involved PCA-LDA. The diagnostic sensitivity was 96.7%, and the specificity was 92%. Further this technique is confined to only in vitro studies.

Fourier-Transform Infrared Spectroscopy

In Vitro Studies

As early as 1995, Wong et al. have compared the two techniques – FTIR and ATR/FTIR spectroscopy. They had analysed exfoliated cells and tissues from human endocervix and ectocervix. The study concludes the effectiveness of the ATR-FTIR over FTIR for thin layers containing different types of cells, as in the case of endocervix. The endocervix sample is mixed with a single layer of columnar cells and the connective tissue. Marked spectral differences in the endo- and ectocervix were observed. The ectocervix show pronounced glycogen bands, whereas in the endocervix, the glycogen bands were not prominent. Even in the case of malignant samples, the glycogen level was found to be lower. Hence they concluded that only the signal strength of glycogen is insufficient to discriminate normal samples from malignant samples [53].

The study carried on by Wood et al. demonstrates the potential of FTIR as a screening tool in clinical setting [54]. The different layers of the cervix epithelium exhibit different spectral signatures. Thus this technique aids in understanding the differentiation and maturation of the epithelium. Analysis of the exfoliated cells in the light of this spectral information yields better perception of the composition and pathological condition of the cells [55, 56].

A number of research groups have extensively studied exfoliated cervical cells employing various multivariate analyses [57–64]. The biochemical changes were detectable before the morphological changes were seen [58]. Neviliappan et al. observed that the spectral features of the cervical adenocarcinoma cell line (SiSo) correlated well with the malignant exfoliated cervical cells and cells from malignant tissues [59]. Diem et al. identified that the cells undergoing proliferation showed prominent spectral features of nucleic acids and phospholipids, while the metabolically inactive cells exhibit signals from proteins [60]. El-Tawil et al. employed the non-parametric Mann-Whitney test of selected biochemical components [62]. The inter-category variance is increased, and the intra-category variance is decreased by multivariate statistical analysis of the IR spectra by employing PCA-LDA [63]. Schubert et al. had developed an algorithm called the Pap Map for processing the infrared spectral data of Pap smear cells. They had demonstrated the efficacy of this technique by analysing blinded samples. They concluded that the spectral cytopathology has the potential to be a new screening technique, based on cellular biochemical changes which can detect the onset of the disease [64].

Cohenford et al. have reported the suitability of employing FTIR for discrimination of normal from dysplastic and malignant cervix tissues [65]. Wong et al. identified the confounding factors in FTIR interpretation. The factors were polymorphs, endocervical columnar cells, metaplastic cells, cervical mucus and debris which led to more of false-positive rates [66]. Romeo et al. addressed the issue of these confounding factors by statistical approach [67]. Mordechai et al. had reported that the intensity ratio of RNA to DNA measured at 1121 and 1020 cm^{-1}, respectively, was higher in malignant samples. They also noted that the glycogen level reduced

considerably in malignant samples [68]. In conjunction with statistical analysis (partial least squares), FTIR spectra were used to detect the presence of biomarkers such as p16INK4A which depends on HPV. This exemplifies the diagnostic potential of FTIR [69].

ATR-FTIR spectroscopy can segregate the different grades of cervical cytology. Hence it reveals the advantages over conventional cervical cytology screening [70]. Classification based on HPV (human papilloma virus) and age has been explored for a better prognosis of the patients [71]. Further identification of the low-grade cervical cytology for the disease progression is aided by ATR-FTIR-based statistical analysis [72]. In addition to this, the technique identifies the presence of atypia or different diseases which are generally missed in conventional cytology [73]. An automated screening system based on feature extraction by discriminant analysis and PCA and the pathological conditions are classified by hybrid multilayered perceptron network. This yielded an accuracy of 92% [74].

Liquid Biopsy

Blood plasma analysed by ATR-FTIR in juxtaposition with multivariate statistical analysis is a promising screening tool for precancerous cervical lesions [75]. Bonnier et al. discussed in detail the spectral analysis of low molecular weight fraction in blood serum [76].

Imaging Modalities

Fluorescence Imaging

Fluorescence Lifetime Imaging Microscopy (FLIM) is also attempted for its efficacy of cervical cancer diagnosis. Gu et al. have proposed a ten-layer analysis of CIN from H&E-stained cervical tissue sections using fluorescence lifetime diagnosis. This study includes 32 H&E-stained cervical tissue sections with all classification (CIN I, II and III) for FLIM analysis. Following the standard CIN diagnosis, the lifetime values were analysed for three-layer epithelial model and, consequent of the obtained results, improved to a ten-layer model and displayed it in false-coloured image. This improved analysis shows the lifetime follows cell maturity. Further the 10-layer analysis proposed by this study betters the sensitivity and specificity values about 1.5 times compared with whole epithelium and 3-layer models [77].

A study using laser scanning confocal microscope was carried out by Pavlova et al. on ten pairs of normal and abnormal biopsied samples. This work was carried out with UV wavelength ranging 351–364 nm and 488 nm excitation before and after addition of the MitoTracker Orange. The cytoplasma and periphery of the cells fluoresce at both excitation wavelengths. The study shows as the epithelium moves from normal to precancerous, enhancement of cytoplasmic fluorescence is observed. This tendency continues with the progression of precancer and correlates well with

the stained sample indicating the increase of mitochondrial density in the course of the progression. The emission properties of stroma are also found to vary with depth. These results of this study equate to the altered biomolecules which explains the variation in emission characteristics of normal and precancerous tissue [28].

Multispectral wide-field microscopic imaging is a technique coupled with diverse optical technologies owing to the advantages like high sensitivity, specificity and resolution. It facilitates high-resolution, real-time images at multiple wavelengths. It can be extended even towards two- and three-photon excitation with close to resolution ranging in microns, coupled with fluorescence and reflectance spectroscopy that can counter the entire field of the cervix [21, 78]. Parker et al. have studied constructing neural net from fluorescence imaging of cervical intraepithelial neoplasia. The results show the correct classification rates 96.5% and 97.5% for the intra-patient and inter-patient, respectively, with a very high sensitivity, specificity and positive and negative predictive values [79].

In a study conducted by Balas [18], in vivo images were obtained with a spectral band of 400–700 nm both before and after the application of acetic acid. Successive spectral analysis reveals the best contrast is in the range of 525 ± 15 nm and is further enhanced by cutting off the tissue reflectance component using polarizers. Snapshot imaging of the sampled area in this spectral band enables quantitative assessment in any spatial location. This study demonstrates the ability to distinguish even between neoplasias of different grades [18]. Chang et al. [80] work highlights that even without biopsy, sectioning or staining, confocal microscopy could be employed to obtain direct in vivo image vital information including altering cellular morphology and N/C ratio [80].

Raman Mapping

Raman mapping is the process by which the entire selected region is scanned stepwise and the spectrum is obtained at every point. Thus obtained large information-rich data set are analysed to glean biological information. Tan et al. had reported Raman mapping of de-paraffinized precancerous cervical tissue sections [81]. The various regions mapped included the squamous epithelium, the epithelial-stromal interface, a muscular artery and endocervical glands. Hierarchical cluster analysis employed here for generating pseudo-colour maps was found to be efficient to discriminate the spectral patterns of different regions though they had residual background due to paraffin wax. The Raman map was able to distinguish the normal region from CIN2. Lori E. Kamemoto et al. in their pilot study have reported the Raman mapping of a malignant cervix tissue [82]. They created two chemical maps by analysing the data set. One Raman map was based on the spectral area under the region 775–975 cm^{-1}. This spectral region was selected since it has the characteristic peaks attributed to collagen. The other Raman map was reconstructed based on the spectral area under 2800–3075 cm^{-1}. Both of these maps had correlated well.

Nosheen Rashid et al. had reported the Raman spectral maps from de-paraffinized cervical tissue samples of 20 different patients, including 5 normal, 2 LSIL (CIN 1),

10 HSIL (5 CIN 2, 5 CIN 3) and 3 carcinoma in situ samples. The spectral data set was subjected to dimension reduction by principal component analysis (PCA). The pseudo-coloured Raman maps were generated based on the cluster membership derived from K-means cluster analysis (KMCA). This study shows that biochemical changes were observed in the normal regions of abnormal samples but morphological changes were not seen [83].

Amuthachelvi Daniel and group have reported the Raman maps from fresh cervical tissues of normal, CIN and malignant samples [84]. They have also employed PCA-KMCA for generating Raman maps. The maps showed clear differences between the different regions of the tissue, and there were spectral changes associated with neoplasia and malignancy. A semi-quantitative biochemical modelling was carried out to quantify these spectral changes and the relative contributions of the biochemicals. Furthermore, the Raman map of the neoplastic tissue revealed that the connective tissue region of the sample was also affected which was not revealed in the standard H&E image. The connective tissue up to 400 μm of the dysplastic sample shows reduced peaks at 853, 918, 935 and 1155 cm^{-1} attributed to glycogen, collagen and elastin (Fig. 3.3). Further this region of connective tissue shows peaks at 722 cm^{-1} and 785 cm^{-1} (DNA: O-P-O, C, U, T, pyrimidine ring breathing mode), 812 cm^{-1} (phosphodiester of DNA), 895 cm^{-1} (the DNA backbone vibration), 1094 cm^{-1} (DNA), 1177 cm^{-1} (C, G), 1335 cm^{-1} (CH_3/CH_2 deforming modes of DNA), 1575 cm^{-1} (G, A and T) and 1654 cm^{-1} (amide I of T, G and C) associated to DNA and RNA. Briefly, due to the inherent sensitivity of Raman spectroscopy, this technique would complement the standard histopathology and reveal information that is otherwise unavailable in it.

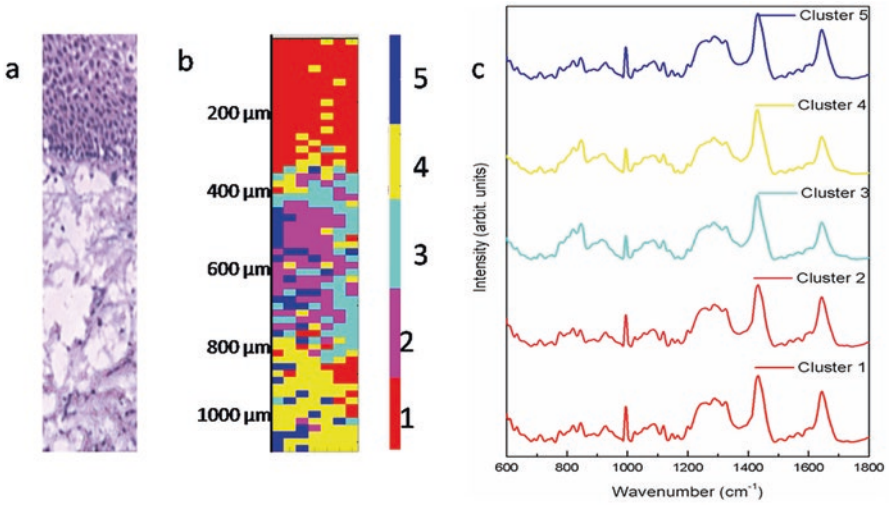

Fig. 3.3 (**a**) H&E image of cervical intraepithelial neoplasia (CIN). (**b**) Corresponding reconstructed Raman image. (**c**) Cluster averages of the Raman spectra collected from the scanned area

Infrared Mapping/Imaging

FTIR mapping or imaging has given a new insight to the molecular-level changes before the onset of morphological changes as seen in H&E image. The spectroscopic signature at the epithelial layer gives profound information about the biochemical composition of the normal and carcinoma in situ (CIS). The epithelial layer of the normal tissue has a significant peak at 1155 cm^{-1}, whereas in CIS, this peak is decreased in intensity, but the intensity of the 1170 cm^{-1} increases [85]. The other significant change observed by Jui-I Chang et al. was the broadening of the band near 1240 cm^{-1} when the disease progresses to CIS. Hence the group has calculated the ratio of the area under the curve at 1130 and 1180 cm^{-1} to the area under the curve at 1180 and 1260 cm^{-1}. This ratio was 1.40 ± 0.91 for normal and − 0.29 ± 0.19 for CIS. The pseudo-colour map was developed based on these ratios.

Wood et al. have studied the spectral features of the different layers of cervix tissue of normal, low-grade and high-grade squamous intraepithelial lesions [86]. They had analysed a whooping 75,000 spectra by unsupervised hierarchical analysis of 1800–800 cm^{-1}. The ecto- and endocervical epithelial layers showed the presence of glycogen and glycoproteins, respectively. The cluster analysis of 1740–1470 cm^{-1} helps in demarcating the squamous epithelial layers because of the variations in the protein bands. The spectral signature of collagen is high in the connective tissue regions. This analysis was able to discriminate the inflammation area. The different regions in LSIL and HSIL were clearly delineated in the FTIR imaging. This is based on the spectral intensity, shape and position of the amide II/amide I band. This band can be attributed to the changes in structure and abundance of protein. Finally, this work has made it obvious that the spectral changes in conjunction with unsupervised hierarchical cluster analysis can discriminate the different layers and diseases of the cervix.

Stellar et al. were the first to perform imaging of squamous cell carcinoma tissue. The tissue was obtained from a radical hysterectomy patient [87]. A 64×64 focal plane array detector was used to obtain 122 chemical images. The spectra were processed first by fuzzy C-means clustering followed by unsupervised hierarchical clustering. The reconstructed Raman images (Fig. 3.4) by analysing the spectral window of 950–1480 cm^{-1} showed the discrimination of cervical stroma, epithelium, inflammation, blood vessels and mucus. Following this, a further analysis of the spectral region of 1420–1480 cm^{-1} was required to distinguish between the different histopathological regions of basal layer, dysplastic lesions and squamous cell carcinoma within the same tissue. This was attributed to the deformation vibration of CH_2 and CH_3 moieties from 1460 to 1452 cm^{-1}. It was observed that the intensity of this band decreased successively from the basal layer to dysplastic tissue and then to squamous cell carcinoma. Hence IR microspectroscopic imaging in conjunction with multivariate analysis yielded a highly specific and sensitive molecular-level classification of the spectra.

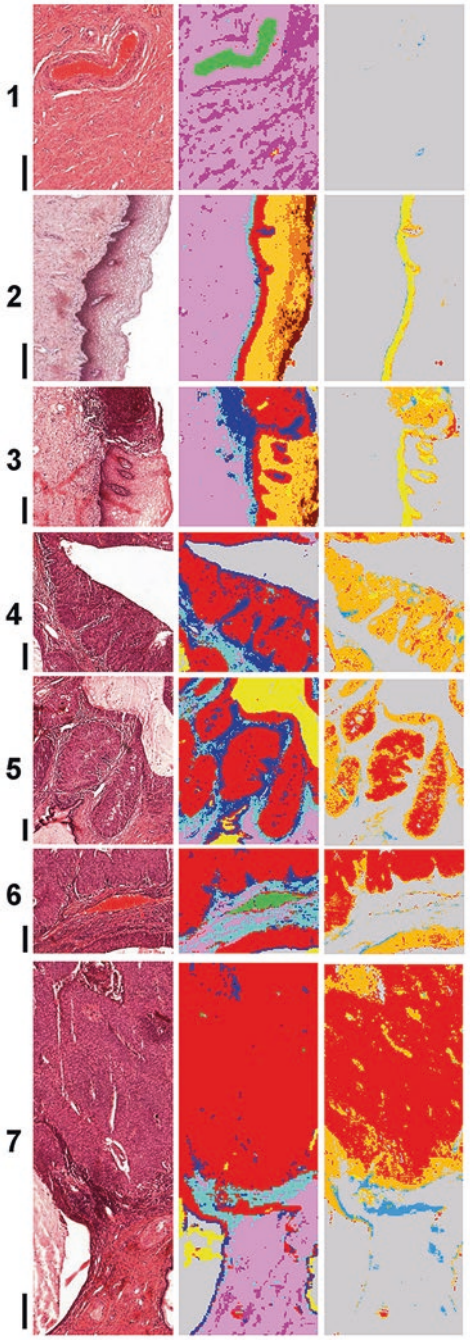

Fig. 3.4 H&E-stained tissue areas 1–7 of Fig. 3.1 (left row), pooled cluster analysis of all IR spectroscopic images in the spectral range 950–1480 cm^{-1} (middle row) and selected cluster analysis of those IR spectra assigned in the pooled cluster analysis to the nucleus-dense areas in the spectral range 1420–1480 cm^{-1} (right row). Bar = 200 µm. Colouring scheme in middle row: nucleus-dense areas of basal cell layer, dysplasia, squamous cell carcinoma (red), parabasal cell layer (light and dark orange), cervical stroma (pink, magenta), inflammation (cyan, blue), mucus (yellow), blood vessel (green). Colouring scheme in right row: basal cell layer (yellow), dysplasia (orange), squamous cell carcinoma (red), non-basal cell layer and non-malignant tissue (blue). (From Steller [87], with permission)

Hyperspectral Imaging

Similarly hyperspectral imaging (HSI) is an emerging imaging modality that uses a three-dimensional data set, with two spatial dimensions and one spectral dimension. The images thus obtained will be spatially resolved and provide valuable information about the tissue physiology, morphology and composition. In a study by Ferries et al., a combination of fluorescence and reflectance spectra is used, and the results show the sensitivity of multimodal HSI to be 97%, against Pap smear's 72% [88], followed by which Benavides et al. created a multispectral imaging digital colposcope with the ability to provide autofluorescence and reflectance images [89].

Conclusion and Future Perspective

Optical spectroscopy and imaging offers rapid, noninvasive or minimally invasive objective diagnosis of cervical cancer as clearly exemplified in this chapter. Furthermore, these optical techniques have proved to have accessed the morphological and biochemical changes associated with oncogenesis. Yet these techniques are still confined to laboratories. A larger global populace are in the developing and underdeveloped countries with the current screening modalities not reaching them. Hence there is an imperative need to develop low-cost, portable, battery-powered optical devices for mass screening. The translation of these techniques to clinical use would involve further research on the discrimination of inflammation and the normal metaplasia changes at squamous columnar junction from normal and precancerous lesions. Further optical multimodal method can also be explored to this end.

References

1. Orfanoudaki IM, Kappou D, Sifakis S. Recent advances in optical imaging for cervical cancer detection. Arch Gynecol Obstet. 2011;284(5):1197–208.
2. Yu B, Shah A, Nagarajan VK, et al. Diffuse reflectance spectroscopy of epithelial tissue with a smart fiber-optic probe. Biomed Opt Express. 2014;5(3):675.
3. Rasooly A, Herold KE. Mobile health technologies. Methods and protocols, vol. 1256. New York: Springer; 2015.
4. Aroca R. Surface-enhaced vibrational spectroscopy. Chichester: Wiley; 2006.
5. Douketis C, Haslett TL, Wang Z, et al. Self-affine silver films and surface-enhanced Raman scattering: linking spectroscopy to morphology. J Chem Phys. 2000;113:11315–23.
6. Baker MJ, Trevisan J, Bassan P, et al. Using Fourier transform IR spectroscopy to analyze biological materials. Nat Protoc. 2014;9(8):1771–91.
7. Van Der Sneppen LS, Ritchie G, Hancock G, et al. Evanescent-wave cavity enhanced spectroscopy as a tool in label-free biosensing. Conference on lasers and electro-optics 2010, OSA technical digest, AMC2. 2010.
8. Mitchell MF, Schottenfeld D, Tortolero-Luna G, et al. Colposcopy for the diagnosis of squamous intraepithelial lesions: a meta-analysis. Obstet Gynecol. 1998;91(4):626–31.

9. Nordstrom RJ, Burke L, Niloff JM, et al. Identification of cervical intraepithelial neoplasia (CIN) using UV-excited fluorescence and diffuse-reflectance tissue spectroscopy. Lasers Surg Med. 2001;29(2):118–27.
10. Mirabal YN, Chang SK, Atkinson EN, et al. Reflectance spectroscopy for in vivo detection of cervical precancer. J Biomed Opt. 2002;7(4):587.
11. Marín NM, Milbourne A, Rhodes H, et al. Diffuse reflectance patterns in cervical spectroscopy. Gynecol Oncol. 2005;99(3 Suppl):116–20.
12. Mourant JR, Bocklage TJ, Powers TM, et al. In vivo light scattering measurements for detection of precancerous conditions of the cervix. Gynecol Oncol. 2007;105(2):439–45.
13. Alvarez RD, Wright TC. Effective cervical neoplasia detection with a novel optical detection system: a randomized trial. Gynecol Oncol. 2007;104(2):281–9.
14. Desantis T, Chakhtoura N, Twiggs L, et al. Spectroscopic imaging as a triage test for cervical disease: a prospective multicenter clinical trial. J Low Genit Tract Dis. 2007;11(1):18–24.
15. Wang A, Nammalavar V, Drezek R. Experimental evaluation of angularly variable fiber geometry for targeting depth-resolved reflectance from layered epithelial tissue phantoms. J Biomed Opt. 2007;12(4):44011.
16. Arifler D, Schwarz RA, Chang SK, Richards-Kortum R. Reflectance spectroscopy for diagnosis of epithelial precancer: model-based analysis of fiber-optic probe designs to resolve spectral information from epithelium and stroma. Appl Opt. 2005;44(20):4291–305.
17. Orfanoudaki IM, Themelis GC, Sifakis SK, et al. A clinical study of optical biopsy of the uterine cervix using a multispectral imaging system. Gynecol Oncol. 2005;96(1):119–31.
18. Balas C. A novel optical imaging method for the early detection, quantitative grading, and mapping of cancerous and precancerous lesions of cervix. IEEE Trans Biomed Eng. 2001;48(1):96–104.
19. Pogue BW, Kaufman HB, Zelenchuk A, et al. Analysis of acetic acid-induced whitening of high-grade squamous intraepithelial lesions. J Biomed Opt. 2001;6(4):397–403.
20. Young Park S, Follen M, Milbourne A, et al. Automated image analysis of digital colposcopy for the detection of cervical neoplasia. J Biomed Opt. 2008;13(1):14029.
21. Thekkek N, Richards-Kortum R. Optical imaging for cervical cancer detection: solutions for a continuing global problem. Nat Rev Cancer. 2008;8(9):725.
22. Collier T, Richards-Kortum R. Real-time reflectance confocal microscopy: comparison of two-dimensional images and three-dimensional image stacks for detection of cervical precancer. J Biomed Opt. 2007;12(2):1–7.
23. Shaikh R, Prabitha VG, Dora TK, et al. A comparative evaluation of diffuse reflectance and Raman spectroscopy in the detection of cervical cancer. J Biophotonics. 2016;11:1–11.
24. Winkelman J, Rasmussen-Taxdal DS. Quantitative determination of porphyrin uptake by tumour tissue following parenteral administration. Bull Johns Hopkins Hosp. 1960;107:228–33.
25. Andersson-Engels S, af Klinteberg C, Svanberg K, Svanberg S. In vivo fluorescence imaging for tissue diagnostics. Phys Med Biol. 1997;42(5):815.
26. Mitchell MF, Cantor SB, Ramanujam N, et al. Fluorescence spectroscopy for diagnosis of squamous intraepithelial lesions of the cervix. Obstet Gynecol. 1999;93(3):462–70.
27. Shahzad A, Edetsberger M, Koehler G. Fluorescence spectroscopy: an emerging excellent diagnostic tool in medical sciences. Appl Spectrosc Rev. 2010;45(1):1–11.
28. Pavlova I, Sokolov K, Drezek R, et al. Microanatomical and biochemical origins of normal and precancerous cervical autofluorescence using laser-scanning fluorescence confocal microscopy. Photochem Photobiol. 2003;77(5):550–5.
29. Drezek R, Brookner C, Pavlova I, et al. Autofluorescence microscopy of fresh cervical-tissue sections reveals alterations in tissue biochemistry with dysplasia. Photochem Photobiol. 2001;73(6):636–41.
30. Ramanujam N. Fluorescence spectroscopy of neoplastic and non-neoplastic tissues. Neoplasia. 2000;2(1–2):89–117.
31. Vaitkuviene A, Gegzna V, Kurtinaitiene R, et al. Cervical smear photodiagnosis by fluorescence. Photomed Laser Surg. 2012;30(5):268–74.

32. Pandey K, Pradhan A, Agarwal A, et al. Fluorescence spectroscopy: a new approach in cervical cancer. J Obstet Gynecol India. 2012;62(4):432–6.
33. Ramanujam N, Mitchell MF, Mahadevan A, et al. Fluorescence spectroscopy: a diagnostic tool for cervical intraepithelial neoplasia (CIN). Gynecol Oncol. 1994;52(1):31–8.
34. Ramanujam N, Mitchell MF, Mahadevan A, et al. Development of a multivariate statistical algorithm to analyze human cervical tissue fluorescence spectra acquired in vivo. Lasers Surg and Med. 1996;19(1):46–62.
35. Mujat C, Greiner C, Baldwin A, et al. Endogenous optical biomarkers of normal and human papillomavirus immortalized epithelial cells. Int J Cancer. 2008;122(2):363–71.
36. Vansevičiūtė R, Venius J, Žukovskaja O, et al. 5-aminolevulinic-acid-based fluorescence spectroscopy and conventional colposcopy for in vivo detection of cervical pre-malignancy. BMC Womens Health. 2015;15(1):35.
37. Zhao Q, He Y, Wang X-L, et al. Differentially expressed proteins among normal cervix, cervical intraepithelial neoplasia and cervical squamous cell carcinoma. Clin Transl Oncol. 2015;17(8):620–31.
38. Mahadevan-Jansen A, Mitchell MF, Ramanujam N, et al. Nearinfrared Raman spectroscopy for in vitro detection of cervical precancers. Photochem Photobiol. 1998;68(1):123–32.
39. Lyng FM, Faolain EO, Conroy J, et al. Vibrational spectroscopy for cervical cancer pathology, from biochemical analysis to diagnostic tool. Exp Mol Pathol. 2007;82(2):121–9.
40. Jess PRT, Smith DDW, Mazilu M, et al. Early detection of cervical neoplasia by Raman spectroscopy. Int J Cancer. 2007;121(12):2723–8.
41. Krishna CM, Prathima NB, Malini R, et al. Raman spectroscopy studies for diagnosis of cancers in human uterine cervix. Vib Spectrosc. 2006;41(1):136–41.
42. Mahadevan-Jansen A, Mitchell MF, Ramanujam N, et al. Development of a fiber optic probe to measure NIR Raman spectra of cervical tissue in vivo. Photochem Photobiol. 1998;68(3):427–31.
43. Utzinger U, Heintzelman DL, Mahadevan-Jansen A, et al. Near-infrared Raman spectroscopy for in vivo detection of cervical precancers. Appl Spectrosc. 2001;55(8):955–9.
44. Robichaux-Viehoever A, Kanter EM, Shappell H, et al. Characterization of Raman spectra measured in vivo for the detection of cervical dysplasia. Appl Spectrosc. 2007;61(9):986–93.
45. Mo J, Zheng W, Low JJH, et al. High wavenumber Raman spectroscopy for in vivo detection of cervical dysplasia. Anal Chem. 2009;81(21):8908–15.
46. Kanter EM, Majumder S, Vargis E, et al. Multiclass discrimination of cervical precancers using Raman spectroscopy. J Raman Spectrosc. 2009;40(2):205–11.
47. Kanter EM, Vargis E, Majumder S, et al. Application of Raman spectroscopy for cervical dysplasia diagnosis. J Biophotonics. 2009;2(1–2):81–90.
48. Kanter EM, Majumder S, Kanter GJ, et al. Effect of hormonal variation on Raman spectra for cervical disease detection. Am J Obstet Gynecol. 2009;200(5):512e1–5.
49. Duraipandian S, Zheng W, Ng J, et al. In vivo diagnosis of cervical precancer using Raman spectroscopy and genetic algorithm techniques. Analyst. 2011;136(20):4328–36.
50. Duraipandian S, Zheng W, Ng J, et al. Simultaneous fingerprint and high-wavenumber confocal Raman spectroscopy enhances early detection of cervical precancer in vivo. Anal Chem. 2012;84(14):5913–9.
51. González-Solís JL, Martínez-Espinosa JC, Torres-González LA, et al. Cervical cancer detection based on serum sample Raman spectroscopy. Lasers Med Sci. 2014;29:979.
52. Li D, Feng S, Huang H, et al. Label-free detection of blood plasma using silver nanoparticle based surface-enhanced Raman spectroscopy for esophageal cancer screening. Analyst. 2013;138(14):3967–74.
53. Wong PTT, Lacelle S, Fung MFK, et al. Characterization of exfoliated cells and tissues from human endocervix and ectocervix by FTIR and ATR/FTIR spectroscopy. Biospectroscopy. 1995;1(5):357–64.
54. Wood BR, Quinn MA, Burden FR, Mcnaughton D. An investigation into FTIR spectroscopy as a biodiagnostic tool for cervical cancer. Biospectroscopy. 1996;2(3):143–53.

55. Chiriboga L, Xie P, Yee H, et al. Infrared spectroscopy of human tissue. I. Differentiation and maturation of epithelial cells in the human cervix. Biospectroscopy. 1998;4:47–53.
56. Chiriboga L, Xie P, Vigorita V, et al. Infrared spectroscopy of human tissue. II. A comparative study of spectra of biopsies of cervical squamous epithelium and of exfoliated cervical cells. Biospectroscopy. 1998;4:55–9.
57. Fung MFK, Senterman MK, Mikhael NZ, et al. Pressure-tuning Fourier transform infrared spectroscopic study of carcinogenesis in human endometrium. Biospectroscopy. 1996;2:155–65.
58. Cohenford MA, Rigas B, et al. Cytologically normal cells from neoplastic cervical samples display extensive structural abnormalities on IR spectroscopy: implications for tumor biology. Proc Natl Acad Sci U S A. 1998;95(26):15327–32.
59. Neviliappan S, Kan LF, Lee Walter TTL, et al. Infrared spectral features of exfoliated cervical cells, cervical adenocarcinoma tissue, and an adenocarcinoma cell line (SiSo). Gynecol Oncol. 2002;85:170–4.
60. Diem M, Chiriboga L, Lasch P, Pacifico A. IR spectra and IR spectralmaps of individual normal and cancerous cells. Biopolym Biospectrosc. 2002;67(4–5):349–53.
61. Sindhuphak R, Issaravanich S, Udomprasertgul V, et al. A new approach for the detection of cervical cancer in Thai women. Gynecol Oncol. 2003;90:10–4.
62. El-Tawil SG, Adnan R, Muhamed ZN, Othman NH. Comparative study between Pap smear cytology and FTIR spectroscopy: a new tool for screening for cervical cancer. Pathology. 2008;40(6):600–3.
63. Walsh MJ, Singh HF, Stringfellow HF, et al. FTIR microspectroscopy coupled with two-class discrimination segregates markers responsible for inter- and intra-category variance in exfoliative cervical cytology. Biomark Insights. 2008;3:179–89.
64. Schubert JM, Bird B, Papamarkakis K, et al. Spectral cytopathology of cervical samples: detecting cellular abnormalities in cytologically normal cells. Lab Investig. 2010;90(7):1068–77.
65. Cohenford MA, Godwin TA, Cahn F, et al. Infrared spectroscopy of normal and abnormal cervical smears: evaluation by principal component analysis. Gynecol Oncol. 1997;66:59–65.
66. Wong PTT, Senterman MK, Jackli P, et al. Detailed account of confounding factors in interpretation of FTIR spectra of exfoliated cervical cells. Biopolymers. 2002;67:376–86.
67. Romeo MJ, Quinn MA, Burden FR, et al. Influence of benign cellular changes in diagnosis of cervical cancer using IR microspectroscopy. Biopolymers. 2002;67:362–6.
68. Mordechai S, Sahu RK, Hammody Z, et al. Possible common biomarkers from FTIR microspectroscopy of cervical cancer and melanoma. J Microsc. 2004;215(1):86–91.
69. Ostrowska KM, Garcia A, Meade AD, et al. Correlation of p16INK4A expression and HPV copy number with cellular FTIR spectroscopic signatures of cervical cancer cells. Analyst. 2011;136:1365–73.
70. Purandare NC, Patel II, Trevisan J, et al. Biospectroscopy insights into the multi-stage process of cervical cancer development: probing for spectral biomarkers in cytology to distinguish grades. Analyst. 2013;138:3909–16.
71. Lima KMG, Gajjar K, Valasoulis G, et al. Classification of cervical cytology for human papilloma virus (HPV) infection using biospectroscopy and variable selection techniques. Anal Methods. 2014;6:9643–52.
72. Purandare NC, Patel II, Lima KMG, et al. Infrared spectroscopy with multivariate analysis segregates low-grade cervical cytology based on likelihood to regress, remain static or progress. Anal Methods. 2014;6:4576–84.
73. Gajjar K, Ahmadzai AA, Valasoulis G, et al. Histology verification demonstrates that biospectroscopy analysis of cervical cytology identifies underlying disease more accurately than conventional screening: removing the confounder of discordance. PLoS One. 2014;9:e82416:1.
74. Yessi Jusman Y, Isa NAM, Ng S-C, et al. Automated cervical precancerous cells screening system based on Fourier transform infrared spectroscopy features. J Biomed Opt. 2016;21(7):075005.
75. Neves ACO, Silva PP, Morais CLM, et al. ATR-FTIR and multivariate analysis as a screening tool for cervical cancer in women from Northeast Brazil: a biospectroscopic approach. RSC Adv. 2016;6:99648–55.

76. Bonnier F, Brachet G, Duong R, et al. Screening the low molecular weight fraction of human serum using ATR-IR spectroscopy. J Biophotonics. 2016;9(10):1085–97.
77. Gu J, Fu CY, Ng BK, et al. Enhancement of early cervical cancer diagnosis with epithelial layer analysis of fluorescence lifetime images. PLoS One. 2015;10(5):1–15.
78. Rowlands CJ, Park D, Bruns OT, et al. Wide- field three-photon excitation in biological samples. Light Sci Appl. 2016;6(5):e16255–9.
79. Parker MF, Mooradian GC, Okimoto GS, et al. Initial neural net construction for the detection of cervical intraepithelial neoplasia by fluorescence imaging. Am J Obstet Gynecol. 2002;187(2):398–402.
80. Chang SK, Arifler D, Drezek R, et al. Analytical model to describe fluorescence spectra of normal and preneoplastic epithelial tissue: comparison with Monte Carlo simulations and clinical measurements. J Biomed Opt. 2004;9(3):511.
81. Tan KM, Herrington CS, Brown CTA. Discrimination of normal from pre-malignant cervical tissue by Raman mapping of de-paraffinized histological tissue sections. J Biophotonics. 2010;4(1–2):40–8.
82. Kamemoto LE, Misra AK, Sharma SK, et al. Near-infrared micro-Raman spectroscopy for in vitro detection of cervical Cancer. Appl Spectrosc. 2010;64(3):255–61.
83. Rashid N, Nawaz H, Poon KWC, et al. Raman microspectroscopy for the early detection of pre-malignant changes in cervical tissue. Exp Mol Pathol. 2014;97(3):554–64.
84. Daniel A, Aruna P, Joseph L, et al. Biochemical assessment of human uterine cervix by micro-Raman mapping. Photodiagn Photodyn Ther. 2017;17:65–74.
85. Chang JI, Huang YB, Wu PC, et al. Characterization of human cervical precancerous tissue through the Fourier transform infrared microscopy with mapping method. Gynecol Oncol. 2003;91:577–83.
86. Wood BR, Chiriboga L, Yee H, et al. Fourier transform infrared (FTIR) spectral mapping of the cervical transformation zone, and dysplastic squamous epithelium. Gynecol Oncol. 2004;93:59–68.
87. Steller W, Einenkel J, Horn L-C, et al. Delimitation of squamous cell cervical carcinoma using infrared microspectroscopic imaging. Anal Bioanal Chem. 2006;384:145–54.
88. Ferris DG, Lawhead RA, Dickman ED, et al. Multimodal hyperspectral imaging for the non-invasive diagnosis of cervical neoplasia. J Low Genit Tract Dis. 2001 Apr;5(2):65–72.
89. Benavides J, Chang S, Park S, et al. Multispectral digital colposcopy for in vivo detection of cervical cancer. Opt Express. 2003 May 19;11(10):1223–36.

Cervical Cancer Screening in Low- and Middle-Income Countries

4

Diama Bhadra Vale, Joana Froes Bragança, and Luiz Carlos Zeferino

Cervical cancer is a disease mainly seen in low- and middle-income countries. According to the World Health Organization, over 80% of new cases arise in those regions [1]. Either breast and cervical cancer are the most common cancers diagnosed in women, and cervical cancer is the most common cancer diagnosis in 45 countries, most of them in developing areas [2]. The cumulative probability of cervical cancer for women aged 15–79 years is less than 1% in high-income regions, while in Mozambique, Zimbabwe, and Eritrea, it peaks at 4.9% [3]. The global cumulative incidence of cervical cancer decreased from 1980 to 2010, although the annual change reduction was lower in developing regions [3].

The World Bank classifies countries by their economies based on their gross national income per capita in low-, middle-, and high-income countries [4]. One striking feature of low- and middle-income countries is the inability to implement sustainable activities to help people reaching some aspects of quality of life. Inequalities in healthcare accessibility lead to a permanent state of suffering and health pauperization to a large number of people. In 2016 the American Society of Cancer Oncology issued a stratified guideline addressing global resource disparities [5]. This initiative highlights the importance of allocating resources to regions of greatest need, in order to achieve a better quality of life for treated women.

Ensuring healthy lives and promoting well-being for all at all ages is the third of the 17 goals of the United Nations "2030 Agenda for Sustainable Development" [6]. Programs for control of cervical cancer are important cost-effectives strategies, along with control of breast cancer and tobacco. Any strategy related to improving cancer control in those regions should aim to reduce inequalities in access to care. The goal of this chapter is to debate challenges from low- and middle-income countries to implement successful cervical cancer screening programs in order to reduce

D. B. Vale (✉) · J. F. Bragança · L. C. Zeferino
Department of Gynecology and Obstetrics, Hospital Dr. José Aristodemo Pinotti, State University of Campinas, Sao Paulo, Brazil
e-mail: dvale@unicamp.br

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_4

cancer burden. The feasibility for introduction of new technologies has to be analyzed within this context.

Cervical Cancer Screening

The long natural history of the cervical cancer makes this neoplasia the one that can more efficiently be prevented by screening. Population-based screening programs have reduced significantly cervical cancer burden in high-income countries over the past few decades [7–9]. In low- and middle-income countries, little reduction in mortality has been observed even in settings where cytology screening is available [10]. A reasonable justification for this difference observed is the main strategy for organization of the programs – whether they are organized or opportunistic. The technology used to screen also may play an important role, but its performance may vary according to the different scenarios.

Screening Strategies: Organization

To constitute a program is desirable, at a minimum, some screening policy documented defining the screening test, the examination intervals, and the group of persons eligible to be screened [11]. It is expected that those recommendations would be based on sufficient evidence and appropriate balances between harms and benefits. The activities to run those policies are the organization strategy for implementation: organized or opportunistic screening.

Organized Screening

"Organized" programs for delivery of screening services generally involve a higher degree of program management. It requires quality control of all steps of the screening process: planning and implementation, coordination of delivery of services, quality control of the tests, invitation, further assessment and follow-up, monitoring by manage of data with adequate links to registries, and generation of reports on performance and others [11].

The cornerstone of an organized screening program is the population-based approach for invitation. The whole target population is personally identified by means of a screening registry and invited usually by letter. The main challenge for low- and middle-income countries is the absence of an individual identification of the target population. This would require resources on public health that goes beyond the implementation of a screening program. Manage with the available demographic data would be an alternative. High-income countries usually use multiples sources to obtain individual data on the population.

Opportunistic Screening

An opportunistic program, in contrast with an organized one, is the one that depends on the subjective individual assessment of risk by the women, as well as the

willingness and ability to participate on screening [12]. In this setting, the program is not able to identify all the target women under risk. Performing the exam usually relies on the access of the women to the healthcare service. Opportunistic screening reaches low coverage, over-screens a small number of women, and is less cost-effective than an organized screening program. The majority of women opportunistically screened do it at shorter intervals than the recommended, and many of them are out of the target age [13, 14].

Some studies have consistently shown differences in participation rates in organized versus opportunistic screening related to socioeconomic characteristics [12, 15]. This may be mainly due to barriers in access and lack of knowledge of women from lower socioeconomic status. Overcoming these aspects, organized screening tends to reduce inequalities in screening programs.

When the program lacks organization, it is difficult to assure that women with abnormal results will have a proper further assessment. Furthermore, it is hard to perform quality assurance of other aspects of the screening process, like quality control of the screening and further assessment, data management, and others. Having a policy written is not enough to overcome these challenges. In Latin America countries, where opportunistic cytology-based screening is widely spread, only a slight decrease on mortality has been observed during the last decades, and cervical cancer is still the second most incident and common cause of cancer death in females [1, 10].

Screening Strategies: Technologies

Cytology-Based Programs

Despite the fact that no randomized controlled trial has examined the cytology test as effective in reducing cervical cancer mortality rates, a significant impact is described in population observational studies in places where it was implemented, particularly in an organized fashion [7–9]. Cytology is a simple to collect, a cheap, and a well-accepted test worldwide. However, its main disadvantage for low- and middle-income countries is to ensure the high complexity quality assurance of the process of running a program based on cytology: collection of an appropriate smear, quality control of the test, and access to the results. Usually those aspects require multiple visits to the women. It's preferable that services would be centralized as much as possible, and a proper data management system is required [16].

One important aspect of cytology-based programs is that several studies have indicated that their sensitivity is low [17, 18]. Liquid-based cytology was introduced in the mid-1990s as a way to improve the performance of the test. Recent studies have shown a better sensitivity to detect high-grade cervical intraepithelial neoplasia compared with the conventional cytology test [19, 20]. The higher cost of this technique is a barrier for its implementation where cytology-based programs are already implemented. However this should be an aim for sophistication of the performance of the program, when resources are available.

Another advantage of using liquid-based cytology instead of primary conventional cytology is facilitation of reflex testing in the residual material that can be tested for the presence of the human papillomavirus in case of borderline/mildly dyskaryotic smears, minimizing the number of visits.

Visual Inspection-Based Programs

Visual inspection methods for cervical cancer prevention are those based on the application on the cervix of Lugol or acid acetic. Those are subjective methods with low accuracy, but rather low infrastructure or training required for its implementation. In low-resource settings, it is very attractive as an approach to initiate a program, as it is associated with a 25–35% reduction in cervical cancer incidence and the frequency of precursor lesions [21]. The main difficulty related with this method is to maintain quality control, once it is a subjective assessment.

The World Health Organization recommends that once a visual inspection with acid acetic (VIA) program is in place, when resources would be available, the program should shift to provide an HPV test. In the absence of a program, VIA alone should be the initial test to start the implementation of a program [22].

HPV-Based Programs

There is now enough evidence from randomized controlled trials that HPV test is more sensitive than cytology to detect precursor lesions of cervical cancer and protects against cervical cancer [23–26]. The main advantage comes from its high negative predictive value allowing longer intervals and assurance of very low risk with negative tests for oncogenic types of HPV [27, 28]. The challenge for low-resource settings of implementing HPV test seems now to be the difficulty of setting up the test within an organized program.

Since the cost-effectiveness of the test comes from its ability to allow longer intervals, there is doubt if this advantage will be maintained in an opportunistic fashion. With no proper control, tests could be repeated at shorter intervals and, in women out of target population, common features in opportunistic programs. However, some studies have published that even once in a lifetime in naïve screening populations, the HPV test can demonstrate impact in reducing the burden of the disease. In this way, even if not sustainable, an opportunistic massive population approach can be pragmatic if high coverage would be achieved.

One problem regardless of the test used is the difficulty to deal with multiple visits during the screening process. In the context of the HPV test, it is expected the development of new technologies with the aim of providing tests with rapid results to allow one single visit for the women, reducing the chance of loss to follow-up. One multicountry recent study that has evaluated the feasibility and performance of a simplified rapid HPV-DNA test, when used for screening women in a see and treat approach (one single visit), found a better sensitivity than for visual inspection with acid acetic or cytology test [29].

Another advantage of the HPV test is the possibility of sampling self-collection. This may be an advantage for communities with cultural barriers to undergo a pelvic exam or even in the absence of a health center facility, particularly in rural and

remotes areas. A study that has complied data from five population-based cervical cancer screening studies in China, involving 13,140 participants, found a sensitivity of self-HPV testing to detect precursor lesions compared favorably with that of LBC and superior to the sensitivity of VIA [30]. Even in settings where the HPV test is already used, self-sampling has demonstrated to increase coverage of non-attendees [31].

Conclusions

- Strategies for implementing and running screening programs should be regionalized, according to the availability of the resources in order to achieve a better quality of life for treated women.
- Cytology-based programs can be effective in settings where it assured adequate control of the tests, access of the results, and further assessment.
- Liquid-based cytology has a better sensitivity to detect high-grade cervical intraepithelial neoplasia than conventional cytology, with higher costs.
- Visual inspection-based programs can be an attractive approach to initiate a cervical cancer screening in low-resource settings.
- HPV test is more sensitive than cytology to detect precursor lesions of cervical cancer and allow longer screening intervals due to high negative predictive value.
- Implementation of screening based on HPV test should be associated to an organized program.

References

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. GLOBOCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC CancerBase No. 11 [Internet]. 2013 [cited 2016 Dec 9]. http://globocan.iarc.fr/Default.aspx
2. Bray F, Jemal A, Grey N, Ferlay J, Forman D. Global cancer transitions according to the Human Development Index (2008–2030): a population-based study. Lancet Oncol. 2012;13(8):790–801.
3. Forouzanfar MH, Foreman KJ, Delossantos AM, Lozano R, Lopez AD, Murray CJL, et al. Breast and cervical cancer in 187 countries between 1980 and 2010: a systematic analysis. Lancet Lond Engl. 2011;378(9801):1461–84.
4. World Bank. How does the World Bank classify countries? – World Bank Data Help Desk [Internet]. [cited 2017 Jun 24]. https://datahelpdesk.worldbank.org/knowledgebase/articles/378834-how-does-the-world-bank-classify-countries
5. American Society of Clinical Oncology. New cervical cancer guideline addresses global resource disparities [Internet]. ASCO. 2016 [cited 2017 Jun 24]. https://www.asco.org/about-asco/press-center/news-releases/new-cervical-cancer-guideline-addresses-global-resource
6. United Nations. Sustainable development goals: 17 goals to transform our world [Internet]. United Nations Sustainable Development. [cited 2017 Jun 24]. http://www.un.org/sustainabledevelopment/

7. Arbyn M, Raifu AO, Weiderpass E, Bray F, Anttila A. Trends of cervical cancer mortality in the member states of the European Union. Eur J Cancer. 2009;45(15):2640–8.
8. Lăără E, Day NE, Hakama M. Trends in mortality from cervical cancer in the Nordic countries: association with organised screening programmes. Lancet. 1987;1(8544):1247–9.
9. Peto J, Gilham C, Fletcher O, Matthews FE. The cervical cancer epidemic that screening has prevented in the UK. Lancet. 2004;364(9430):249–56.
10. Murillo R, Almonte M, Pereira A, Ferrer E, Gamboa OA, Jerónimo J, et al. Cervical cancer screening programs in Latin America and the Caribbean. Vaccine. 2008;26:L37–48.
11. Ponti A, et al. Cancer screening in the European Union: second report on implementation [Internet]. [cited 2017 Apr 24]. https://ec.europa.eu/health/sites/health/files/major_chronic_diseases/docs/2017_cancerscreening_2ndreportimplementation_en.pdf
12. Walsh B, Silles M, O'Neill C. The importance of socio-economic variables in cancer screening participation: a comparison between population-based and opportunistic screening in the EU-15. Health Policy Amst Neth. 2011;101(3):269–76.
13. Adab P, McGhee SM, Yanova J, Wong CM, Hedley AJ. Effectiveness and efficiency of opportunistic cervical cancer screening: comparison with organized screening. Med Care. 2004;42(6):600–9.
14. do Vale DBAP, Morais SS, Pimenta AL, Zeferino LC. Assessment of the cervical cancer screening in the Family Health Strategy in Amparo, São Paulo State, Brazil. Cad Saude Publica. 2010;26(2):383–90.
15. Palència L, Espelt A, Rodríguez-Sanz M, Puigpinós R, Pons-Vigués M, Pasarín MI, et al. Socio-economic inequalities in breast and cervical cancer screening practices in Europe: influence of the type of screening program. Int J Epidemiol. 2010;39(3):757–65.
16. Miller AB, Nazeer S, Fonn S, Brandup-Lukanow A, Rehman R, Cronje H, et al. Report on consensus conference on cervical cancer screening and management. Int J Cancer. 2000;86(3):440–7.
17. Sankaranarayanan R, Gaffikin L, Jacob M, Sellors J, Robles S. A critical assessment of screening methods for cervical neoplasia. Int J Gynaecol Obstet Off Organ Int Fed Gynaecol Obstet. 2005;89(Suppl 2):S4–12.
18. International Agency for Research on Cancer. IARC handbooks of cancer prevention: cervix cancer screening. Vol. 10. IARC; 2005.
19. Rozemeijer K, Naber SK, Penning C, Overbeek LIH, Looman CWN, de Kok IMCM, et al. Cervical cancer incidence after normal cytological sample in routine screening using SurePath, ThinPrep, and conventional cytology: population based study. BMJ. 2017;356:j504.
20. Rozemeijer K, Penning C, Siebers AG, Naber SK, Matthijsse SM, van Ballegooijen M, et al. Comparing SurePath, ThinPrep, and conventional cytology as primary test method: SurePath is associated with increased CIN II+ detection rates. Cancer Causes Control. 2016;27(1):15–25.
21. Sankaranarayanan R, Nessa A, Esmy PO, Dangou J-M. Visual inspection methods for cervical cancer prevention. Best Pract Res Clin Obstet Gynaecol. 2012;26(2):221–32.
22. WHO. Guidelines for screening and treatment of precancerous lesions for cervical cancer prevention [Internet]. WHO. [cited 2015 Aug 11]. http://www.who.int/reproductivehealth/publications/cancers/screening_and_treatment_of_precancerous_lesions/en/
23. Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Dalla Palma P, Del Mistro A, et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncol. 2010;11(3):249–57.
24. Rijkaart DC, Berkhof J, Rozendaal L, van Kemenade FJ, Bulkmans NWJ, Heideman DAM, et al. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. Lancet Oncol. 2012;13(1):78–88.
25. Castle PE, Stoler MH, Wright TC, Sharma A, Wright TL, Behrens CM. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. Lancet Oncol. 2011;12(9):880–90.

26. Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM, et al. HPV screening for cervical cancer in rural India. N Engl J Med. 2009;360(14):1385–94.
27. Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, Scott DR, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst. 2005;97(14):1072–9.
28. Dillner J, Rebolj M, Birembaut P, Petry K-U, Szarewski A, Munk C, et al. Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study. BMJ. 2008;337:a1754.
29. Jeronimo J, Bansil P, Lim J, Peck R, Paul P, Amador JJ, et al. A multicountry evaluation of careHPV testing, visual inspection with acetic acid, and Papanicolaou testing for the detection of cervical cancer. Int J Gynecol Cancer. 2014;24(3):576–85.
30. Zhao F-H, Lewkowitz AK, Chen F, Lin MJ, Hu S-Y, Zhang X, et al. Pooled analysis of a self-sampling HPV DNA test as a cervical cancer primary screening method. J Natl Cancer Inst. 2012;104(3):178–88.
31. Gök M, van Kemenade FJ, Heideman DAM, Berkhof J, Rozendaal L, Spruyt JWM, et al. Experience with high-risk human papillomavirus testing on vaginal brush-based self-samples of non-attendees of the cervical screening program. Int J Cancer. 2012;130(5):1128–35.

Pathology and Molecular Diagnosis of Cervical Cancer and Precursor Lesions

5

Mariana Canepa, Nimesh R. Patel, and Maria Luisa Garcia-Moliner

Pathologic diagnosis of cervical neoplasia incorporates the practice of cytopathology, molecular pathology, and surgical pathology. Cervical pathology includes preinvasive squamous lesions (squamous intraepithelial lesions/SIL), invasive squamous cell carcinoma, adenocarcinoma in situ, invasive adenocarcinoma, as well as other less common primary epithelial and mesenchymal tumors. The most common cervical lesions are human papillomavirus (HPV)-associated squamous lesions. A two-tiered diagnostic approach classifying preinvasive lesions into low-grade and high-grade squamous intraepithelial lesions is used in cytopathology and surgical pathology. Low-grade squamous intraepithelial lesions (LSIL), which include condylomata, are the result of productive HPV infection that may be transient and regress. High-grade squamous intraepithelial lesions (HSIL) have a greater risk of progression to invasive carcinoma and require further treatment. Adenocarcinoma in situ, a precursor to invasive adenocarcinoma, is also HPV-associated and may coexist with squamous lesions. HPV testing/genotyping has been incorporated into the Papanicolaou cytology screening and helps stratify patients into those needing further evaluation. The incidence of invasive carcinoma has decreased with the widespread use of cytology testing. A subset of tumors not associated with HPV infection, while rare, may present diagnostic challenges, especially in cytology and small biopsies. The WHO classification of cervical tumors is presented in Table 5.1 [1].

M. Canepa (✉) · N. R. Patel · M. L. Garcia-Moliner
Department of Pathology and Laboratory Medicine, Brown University Warren Alpert Medical School, Providence, RI, USA
e-mail: mcanepa1@lifespan.org; nimesh.patel@lifespan.org; mgarciamoliner@lifespan.org

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_5

Table 5.1 WHO classification of tumors of the uterine cervix

Epithelial tumors
Squamous cell tumors and precursors
Squamous intraepithelial lesions
Low-grade squamous intraepithelial lesion
High-grade squamous intraepithelial lesion
Squamous cell carcinoma, NOS
Keratinizing
Nonkeratinizing
Papillary
Basaloid
Warty
Verrucous
Squamotransitional
Lymphoepithelioma-like
Benign squamous cell lesions
Squamous metaplasia
Condyloma acuminatum
Squamous papilloma
Transitional metaplasia
Glandular tumors and precursors
Adenocarcinoma in situ
Adenocarcinoma
Endocervical adenocarcinoma, usual type
Mucinous adenocarcinoma, NOS
Gastric type
Intestinal type
Signet ring cell type
Villoglandular carcinoma
Endometrioid adenocarcinoma
Clear cell adenocarcinoma
Serous adenocarcinoma
Mesonephric carcinoma
Adenocarcinoma admixed with neuroendocrine carcinoma
Benign glandular tumors and tumorlike lesions
Endocervical polyp
Müllerian papilloma
Nabothian cyst
Tunnel clusters
Microglandular hyperplasia
Lobular endocervical glandular hyperplasia
Diffuse laminar endocervical hyperplasia
Diffuse laminar endocervical hyperplasia
Mesonephric remnants and hyperplasia
Arias-Stella reaction
Endocervicosis

(continued)

Table 5.1 (continued)

Endometriosis
Tuboendometrioid metaplasia
Ectopic prostate tissue
Other epithelial tumors
Adenosquamous carcinoma
Glassy cell carcinoma
Adenoid cystic carcinoma
Adenoid basal carcinoma
Neuroendocrine tumors
Low-grade neuroendocrine tumor
Carcinoid tumor
Atypical carcinoid tumor
High-grade neuroendocrine tumor
Small cell neuroendocrine carcinoma
Large cell neuroendocrine carcinoma
Undifferentiated carcinoma
Mesenchymal tumors and tumorlike conditions
Benign
Leiomyoma
Rhabdomyoma
Others
Malignant
Leiomyosarcoma
Rhabdomyosarcoma
Alveolar soft-part sarcoma
Angiosarcoma
Malignant peripheral nerve sheath tumor
Other sarcomas
Liposarcoma
Undifferentiated endocervical sarcoma
Ewing sarcoma
Tumorlike lesions
Postoperative spindle cell nodule
Lymphoma-like lesion
Mixed epithelial and mesenchymal tumors
Carcinosarcoma (malignant Müllerian mixed tumor)
Adenosarcoma
Adenomyoma
Melanocytic tumors
Malignant melanoma
Blue nevus
Germ cell tumors
Yolk sac tumor
Lymphoid and myeloid tumors

(continued)

Table 5.1 (continued)

Lymphomas
Myeloid neoplasms
Secondary tumors

From Kurman et al. [1], with permission

HPV and Cervical Dysplasia/Neoplasia (Biology and Pathogenesis)

Human papillomaviruses (HPVs) are non-enveloped, icosahedral DNA viruses that belong to the *Papillomaviridae* family and infect skin and mucous membranes. HPVs may give rise to condylomata as well as to cervical, vaginal, vulvar, penile, anal, and oropharyngeal carcinoma. HPV is the major risk factor for the development of cervical cancer.

More than 200 HPV genotypes have been identified [2]. Approximately 40 HPV types are common in the anogenital tract [3], including the cervix, and can be spread through direct sexual transmission. HPV is classified into low- and high-risk types. High-risk types are those that are most frequently identified in premalignant and malignant lesions, whereas low-risk types are rarely associated with these lesions [4]. HPV infection, particularly with high-risk HPV (HR HPV), is common; however, most infections are cleared within 12–18 months [5]. Persistent infection with HR HPV is associated with the development of cervical carcinoma and precursor lesions.

Productive infection with low-risk HPV (LR HPV), such as types 6 and 11, causes anogenital condylomata [6]. In contrast, HR HPV types have evolved the ability to persist at certain sites of infection and to drive cell proliferation in the basal and parabasal cell layers [7]. As a result, HR HPVs are causally associated with the development of cervical cancer [3, 6], with HPV types 16 and 18 responsible for 70% of cases [6, 8]. HR HPV testing may be used as an adjunct to cytology for cervical cancer screening. Typically, 14 HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) are included in clinical assays due to their oncogenic potential [3].

HPVs have a circular double-stranded DNA genome of about 8 kilobases in length [3]. This consists of an upstream noncoding region (long control region, LCR), which regulates transcription, and eight overlapping open reading frames.

HPV infects the basal cell layer of the cervical epithelium. As the infected cells divide, the virus spreads from the basal layer to the differentiated superficial epithelium. The HPV open reading frames are divided into six early (E) and two late (L) genes, named according to their pattern of expression during this process.

E1, E2, E5, E6, and E7 are expressed early on, playing a role in cell proliferation and genome maintenance, while E4 is expressed throughout differentiation [7]. In contrast, L1 and L2, which encode capsid proteins [3, 7], are only expressed in differentiated surface squamous cells during the late stage of productive infections [7].

L1 is the most highly conserved gene, but differences in L1 can be used to differentiate HPV types [4].

Once infection occurs in the basal layer, viral genome expression can be suppressed, leading to a "silent infection." Cell-mediated immune response may lead to viral clearance or to viral latency without life cycle completion. Alternatively, there can be ordered viral gene expression with viral synthesis and release from the upper epithelial layers (productive infection) or deregulated viral gene expression with the development of preneoplastic lesions. Persistent infection with HR HPV is associated with an increasing risk of integration of the viral genome into host cell chromosomes and progression to cancer [9].

Cervical infection with HPV is thought to require access of the virus to the cells of the squamocolumnar junction, where most precursor lesions arise [10], and specifically to the immature basal epithelial cell layer. This may be related to increased accessibility and proliferation of the basal cell layers at this metaplastic epithelial site [9]. Microscopic tears in the exocervical mucosa also allow the virus to gain access to germinal cells in the basal stem cell layer [4, 7]. The immature metaplastic cells and glandular cells at the thin squamocolumnar junction are also a target of HPV [9].

The virus is taken up by cells through endocytosis, and the viral DNA is transported into the nucleus for transcription and replication [4]. Once inside the host cell, HPV DNA replicates as the basal cells differentiate and progress to the epithelial surface [11]. HPV replication begins when viral and host factors interact with the LCR region of the HPV genome and begin transcription of the viral E6 and E7 genes [11]. Initially, the DNA is maintained in an episomal form in the basal cell layers at a relatively low copy number [4]. Episomal DNA may integrate into or exist independently of the host chromosomal DNA.

In the differentiated keratinocytes of the suprabasal layers of the epithelium, the virus amplifies its DNA to high copy number, synthesizes capsid proteins, and causes viral assembly to occur [11]. Active replication of host cells allows for replication of the viral episomal DNA within the infected cell, as the virus takes advantage of the host cell machinery to replicate. In productive infections, the HPV genome is maintained in an episomal form by the E1 protein, and E2 suppresses the E6/E7 promoter region [4]. If the E1 protein is not fully expressed, the viral genome is incorporated into the host DNA, resulting in loss or fragmentation of genes, such as E4, E5, and E2 [4]. The loss or disruption of E2 results in upregulation of E6 and E7 [4].

Oncoproteins E6 and E7 are of particular importance because they interact with host proteins to stimulate cell cycle progression (enabling viral replication in typically quiescent epithelial layers) and contribute to genomic instability within the host cell [3, 4]. E6 and E7 inhibit the action of the tumor suppressor proteins p53 and pRb (retinoblastoma protein), respectively. E6 inhibits p53 blockage of apoptosis, while E7 inactivates pRb, an important cell cycle regulator [4, 8]. By interfering with cell cycle checks on excessive growth, this process can help the infected cell avoid apoptosis and grow in an uncontrolled fashion. In this manner, overexpression

of E6 and E7 can be associated with an accumulation of genetic changes that eventually leads to carcinoma [9].

The p53 tumor suppressor protein has numerous functions in the cell, including responding to DNA damage by causing the cell to arrest in G_1 phase, as well as inducing apoptosis in damaged cells [4]. The E6 protein of HPV binds to p53 from the host cell and stimulates its degradation through the ubiquitin-dependent protease system, thereby abrogating its functional effects. E6 also inactivates other pro-apoptotic proteins and promotes maintenance of telomere length, thus preventing death of infected cells [4].

The HPV E7 oncoprotein inhibits the function of another tumor suppressor protein crucial for cell cycle regulation, pRb. During the cell cycle, cyclin-dependent kinases control passage from G_1 to the S (DNA synthesis) phase of the cell cycle in order to regulate DNA replication. In response to extracellular signals, Cyclin D interacts with cyclin-dependent kinases and phosphorylate pRb, inactivating it and resulting in an irreversible commitment of the cell to enter S phase and replicate [4]. INK4 proteins, such as $p16^{INK4a}$, inhibit cyclin D-dependent kinases CDK4 and CDK6; in turn, pRb usually acts as a negative regulator of $p16^{INK4a}$ expression [4]. HPV protein E7 is similar in structure and function to Cyclin D1 and, consequently, regulates cellular proliferation by inactivating pRb and interacting with other proteins involved in proliferation [4].

In infection with LR HPV, basal cell proliferation is primarily driven by growth factors in a manner similar to uninfected epithelium; in the upper epithelial layers, the HPV E6 and E7 proteins stimulate cell cycle entry, but not cell proliferation, leading to amplification of the genome [9]. In HR HPV infection, E6 and E7 expression additionally stimulates cell cycle entry and proliferation in the lower and middle layers of the epithelium, driving neoplasia [9]. The E6 and E7 gene products of LR HPV have decreased affinity for p53 and pRb, as well as other functional differences from their HR HPV counterparts [9], helping to explain the different outcomes of these HPV types.

In productive infections, genome amplification occurs in the more superficial layers of the squamous epithelium [8]. In these differentiated cells, expression of the late genes L1 and L2 results in capsid formation, and virions are ultimately assembled [3]. The virions are shed in desquamating cells, but this process is non-lytic, and, therefore, the virus is protected from the host immune system [3].

Molecular Diagnosis of Cervical Neoplasia

HR HPV testing has been recommended as an adjunct to cervical cytology screening (co-testing/reflex testing) or as a primary screening modality [12, 13]. General HR HPV assays detect the presence of one or more of a pool of 13 or 14 oncogenic HPV types without identifying the specific type. The majority of cervical carcinomas and precursor lesions are caused by HPV 16 or 18, and persistent infection with these subtypes has been associated with increased risk of cervical neoplasia [14,

15]. Additional genotyping for these types may be used for further risk stratification of HR HPV-positive women [14, 15].

Over 100 tests have been established for the detection of HPV [16], and, in recent years, numerous commercial assays have been developed for clinical use. Assays can be performed on several specimen types – most commonly, in those obtained for liquid-based cytology examination. Detection of HPV DNA in cervical tissue samples can indicate a variety of conditions, including infection, recent transmission without infection, latent, or silent infection. Since the availability of assays continues to evolve, the general principles of some of the more common assays [17–19] will be discussed, and some of the more commonly used examples are listed in Table 5.2.

HR HPV testing may be performed by a variety of qualitative molecular techniques, such as amplification of portions of the viral genome by polymerase chain reaction (PCR).

The Roche cobas® HPV tests utilize PCR-based amplification of target DNA and nucleic acid hybridization for the detection of 14 HR HPV types in a single analysis. Real-time PCR is performed to amplify a polymorphic region of L1 in the HPV genome. By using a cocktail of primers and differentially labeled fluorescent probes, the assay provides specific detection of HPV 16, HPV 18, and a pool of other HR HPV types.

In contrast to this methodology, other assays capture viral DNA or RNA from specimens using probes. Hybridization results in generation of a signal, which may be amplified to aid in detection. The Qiagen Hybrid Capture® 2 utilizes RNA probes that hybridize with the DNA of 13 HR HPV types [16]. These probes are not designed to distinguish between specific HPV types in a given specimen. In a second capture step, the RNA/DNA hybrids are themselves captured on an antibody-coated plate. Finally, the immobilized hybrids react with additional antibodies that undergo a chemical reaction (chemiluminescence). This results in a light signal that indirectly indicates the presence of HPV DNA [16]. The signal is amplified since multiple antibodies bind to each captured RNA/DNA hybrid.

In addition to DNA markers of viral gene expression, mRNA and proteins can be detected. The Hologic Aptima® HPV assay targets HR HPV mRNA from the E6 and E7 oncogenes, the expression of which is associated with incorporation of the viral genome into the host DNA. In this assay, the target mRNA is captured, amplified, and then detected using chemiluminescence. Similar to other commercial assays, the general HR HPV Aptima assay can be paired with a specific typing assay that can be used as a reflex for positive HR HPV samples. The Aptima® HPV 16 18/45 genotype assay is a complementary test which uses light emission kinetics to differentiate HPV 16 from HPV 18 and/or HPV 45. However, it does not differentiate between HPV types 18 and 45.

HR HPV may also be identified in tissue sections by in situ hybridization (ISH), which allows for the detection of nucleic acids within intact cells and correlation and morphologic changes. Nucleic acid probes specific for HPV DNA or mRNA are labeled with chemically reactive ligands that can be detected by light or fluorescent

Table 5.2 Summary of selected HPV assays

Assay	Assay type	Target	General vs specific genotype
Roche cobas®	Real-time PCR	DNA	Specific (HPV 16 and 18) and general (12 other HR HPV)
Qiagen Hybrid Capture® 2	Capture probe with signal amplification	DNA	General (13 HR HPV types)
Hologic Cervista™ HPV	Invader Chemistry™(signal amplification)	DNA	General (14 HR HPV types)
Hologic Cervista™ HPV 16/18	Invader Chemistry™ (signal amplification)	DNA	Specific (HPV 16 and 18)
Hologic Aptima® HPV	Capture probe with signal amplification	RNA	General
Hologic Aptima® HPV 16/18 45	Capture probe with signal amplification	RNA	Specific (HPV 16 or 18/45)
In situ hybridization	Tissue-based hybridization of target with probe	DNA/ RNA	General
Immunohistochemistry	Antibody detection of protein	Protein	General

Data from Refs. [17–19]

microscopy, depending on the type of probe. DNA and more recently mRNA ISH have demonstrated high sensitivity for the detection of HR HPV [13].

Squamous Lesions of the Cervix

Squamous Intraepithelial Lesion

Surgical Pathology

Cervical squamous neoplasia results from HPV infection. Various terminologies (Table 5.3) have been used in the diagnosis of noninvasive squamous neoplasia, including dysplasia, cervical intraepithelial neoplasia (CIN), and squamous intraepithelial lesion (SIL). Dysplasia – graded as mild, moderate, and severe – is equivalent to CIN 1, 2, and 3, respectively. Squamous cell carcinoma in situ (CIS) is included in severe dysplasia/CIN3. The SIL terminology is two-tiered: low- and high-grade SIL (LSIL, HSIL). LSIL encompasses CIN1 and HPV cytopathic effect/condyloma, and HSIL is comprised of CIN2 and CIN3. SIL, the longstanding terminology of cytology specimens [20], is being increasingly used for cervical surgical specimens, as well as for HPV-related lesions of the rest of the anogenital tract [21].

SIL is the terminology recommended by the WHO as it reflects the biology of HPV disease and provides therapeutic guidance [1]. LSIL, the result of productive infection with low- or high-risk HPV [9], is likely to regress and may be followed without further intervention [22]. HSIL, the result of abortive infection with

Table 5.3 Classification of squamous intraepithelial lesions of the cervix by the different terminologies

Terminology			
Dysplasia	Mild	Moderate	Severe
CIN	1	2	3
SIL	LSIL	HSIL	HSIL

high-risk HPV [9], has a greater risk of progression to invasive carcinoma and requires further treatment [22].

While HR HPV may give rise to HSIL, most HR HPV infections give rise to LSIL. In LSIL, there is a proliferation of immature basal/parabasal cells that is limited to the lower one-third of the epithelium. Mitotic activity may be increased but is also limited to the lower one-third of the epithelial thickness. The upper two-thirds of the epithelium show cellular maturation with mild increase in the nuclear-to-cytoplasmic ratio, binucleated cells, and koilocytes (Fig. 5.1a). Koilocytes, characteristic of HPV cytopathic effect, have hyperchromatic nuclei, with irregular nuclear contours surrounded by a clear cytoplasmic halo. The halo results from E4-driven disruption of cytokeratin with condensation of tonofilaments at the periphery of the cytoplasm of infected cells [4]. LSIL may involve endocervical glands.

Condyloma acuminatum, a variant of LSIL most often seen in the lower anogenital tract, is typically the result of infection with LR HPV (HPV 6 and 11) [23]. The defining feature of condyloma is its warty architecture which can be seen grossly. Microscopically, it is characterized by papillomatous fronds with fibrovascular cores and HPV cytopathic effect.

HSIL arises at the cervical squamocolumnar junction and is characterized by greater cytological atypia than that seen in LSIL (Fig. 5.1b). Hyperchromatic cells with increased nuclear-to-cytoplasmic ratio fail to differentiate as they approach the surface. Loss of epithelial polarity imparts a disorganized appearance to the epithelium. Mitotic figures, including abnormal mitosis, may be seen throughout the epithelial thickness. In CIN 3, these changes encompass more than two-thirds of the epithelial thickness. In CIN 2, the changes occupy between one- and two-thirds of the epithelial thickness; and some degree of cytoplasmic differentiation, including koilocytosis, is seen in the superficial epithelial layers. Similar changes may involve the underlying endocervical glands.

The diagnosis of SIL on cervical biopsies is not always straightforward. The intra- and interobserver variability in the diagnosis of SIL has been well-documented [21, 24–27]. The intermediate category of CIN 2 has the poorest reproducibility. It is now thought that CIN 2 is an equivocal diagnosis likely representing a mixture of CIN 1 and CIN 3. This is reflected in the variable outcomes of CIN2, with some lesions regressing and others progressing [21, 25–29]. Another clinically relevant distinction is between SIL, specifically HSIL, and benign mimics, such as reactive or reparative changes in the setting of infection or inflammation, immature squamous metaplasia, and changes associated with atrophy.

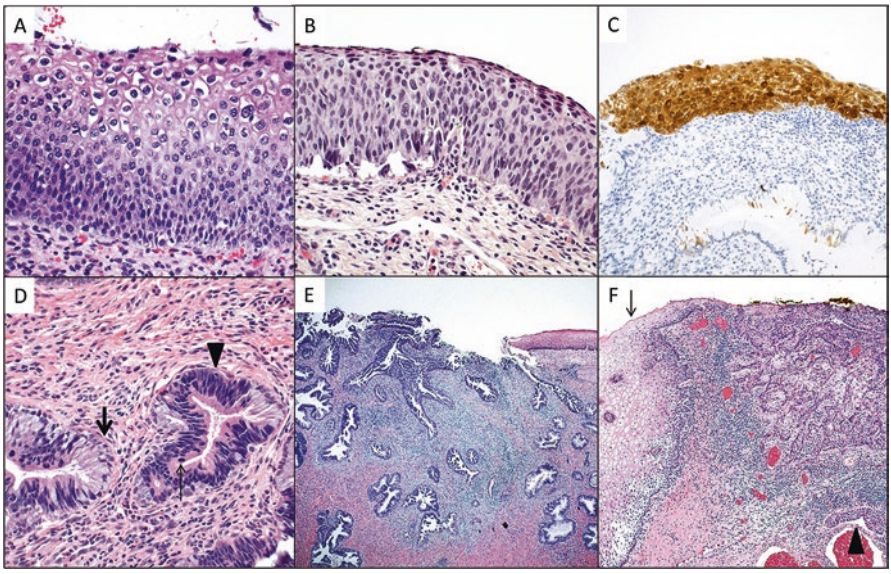

Fig. 5.1 Abnormal findings in cervical biopsy. (**a**) LSIL: enlarged hyperchromatic cells are more prominent in the lower third of the epithelium. Binucleated cells and koilocytes are present on the surface (H&E 400X). (**b**) HSIL: disordered cells with increased nuclear-to-cytoplasmic ratio occupy the entire thickness of the epithelium (H&E 400X). No koilocytes are identified in this example of CIN3. (**c**) HSIL: p16 immunostain shows diffuse and strong positivity. (**d**) AIS: hyperchromatic, pseudostratified cells with mitoses (thin arrow) and apoptotic bodies (arrowhead). Residual normal endocervical cells are also appreciated (thick arrow). (**e**) Invasive endocervical adenocarcinoma: irregular infiltrating glands surrounded by desmoplastic stroma and inflammatory cells. Normal squamous epithelium present on the right upper corner. (**f**) Invasive squamous cell carcinoma: irregularly infiltrating nests of neoplastic squamous cells. Normal epithelium on the left (arrowhead) and lymphatic vessel invasion at the bottom right (arrow)

The use of biomarkers to allow a more objective diagnosis of SIL has been the object of numerous studies [28, 30–33]. The most widely used biomarker is the $p16^{INK4a}$ antibody (p16), a marker of HR HPV E7-driven cell proliferation. Overexpression of E7 in HR HPV infections results in degradation of pRb [4], terminating the negative feedback on the cyclin-dependent kinase inhibitor p16 and leading to overexpression of p16. Increased p16 expression can therefore be used as a surrogate marker of HR HPV infection [3].

Use of p16 immunohistochemistry, alone or in combination with the proliferation marker Ki-67, has been shown to be helpful in making the distinction between SIL and its benign mimics [28, 33–36]. Diffuse, strong staining of the basal and parabasal epithelium ("block" positivity) with p16 is characteristic of HSIL (Fig. 5.1c) and is observed in a variable number of LSIL [33]. Mimics of HSIL, including reactive changes, immature metaplasia, and atrophy, are negative for p16. Staining of occasional cells with the p16 antibody is considered negative, as this is a non-specific finding or may be seen with LSIL.

p16 immunostaining is not recommended for routine grading of SIL [21]. However, p16 may be helpful in difficult cases when the diagnosis of LSIL vs HSIL (CIN2) is being considered [28, 33, 35, 37]. Lack of diffuse p16 staining would support a diagnosis of LSIL. Diffuse p16 staining is less helpful in such situations as a variable number (40% in a meta-analysis) [33] of LSIL are positive for p16. The role of p16 as a predictor of progression of LSIL has not been established [38–41].

Ki-67 immunostaining, which identifies proliferating cells, is particularly helpful in distinguishing HSIL from atrophy. Atrophic squamous epithelium shows a monotonous population of cells with high nuclear-to-cytoplasmic ratios replacing the entire thickness of the epithelium. The lack of surface differentiation may mimic HSIL. Ki-67 immunostaining is increased in SIL, where proliferating cells are present in the upper epithelial layers. In contrast, few or no basal/parabasal cells stain with Ki-67 in atrophic epithelium. Increased Ki-67 staining may also be seen in reactive epithelium, as well as in the inflammatory cells that may infiltrate the epithelium under reactive conditions. ProExC, another proliferation marker, shows a similar pattern of staining as Ki-67 [36].

Cytology

Good cellular detail is essential for the interpretation of cervical cytological specimens or Papanicolaou (pap) smears. Two methods of preparation are in widespread use: direct smears and liquid-based methods.

In direct smears, exfoliated cervical cells are spread on a glass slide, followed by immediate fixation in ethanol. In liquid-based methods, the exfoliated cells are placed directly on a vial containing an alcohol-based preservative.

When performed correctly, direct smears and liquid-based cytology are equivalent methods for detecting cervical pathology [42, 43].

Advantages of direct smears include the low cost to make and process the slides. However, the quality of the smears is operator-dependent, and blood and inflammation can obscure cellular detail, resulting in a higher unsatisfactory rate than liquid-based methods [44].

Liquid-based methods require expensive supplies (collection kit) and laboratory equipment [45]. Processing involves automated instrumentation (ThinPrep® Hologic, Inc., Bedford, MA; SurePath ™ BD Diagnostics, Durham, NC) to create a monolayer sheet of cells. Aliquots from the sample can be used for HPV molecular testing. Obscuring inflammatory cells and debris that may interfere with interpretation are cleared. Liquid-based methods allow for computer-assisted analysis of the samples. While all slides flagged as abnormal by the automated screening require review by a cytotechnologist or pathologist, the process increases the laboratory's productivity. Because of these advantages, liquid-based cytology has virtually replaced conventional pap smears in the United States [46].

The optimal time to obtain a cervical sample is during the proliferative phase of the menstrual cycle, at least 5 days after the end of the menstrual period [47]. Menstrual blood obscures cellular detail in conventional pap smears. Though the red blood cells may be lysed during processing of liquid-based cytology, shed endometrial cells may be confused with glandular neoplasia.

Sample adequacy is determined by quantitative and qualitative criteria. Adequate cellularity requires at least 5000 nucleated squamous cells for liquid-based methods and 8000–12,000 nucleated squamous cells in a conventional smear [20]. At least 75% of the cells must be well-visualized without interfering factors such as blood, mucus, or debris [20]. A statement of specimen adequacy is part of *The Bethesda System for Reporting Cervical Cytology* (see below) [20]. An endocervical glandular component or squamous metaplastic cells imply sampling of the transformation zone/squamocolumnar junction, where most dysplasias arise. Lack of endocervical or squamous metaplastic cells shortens the screening interval, but does not render a specimen unsatisfactory.

Standardized reporting of cervical cytology allows for clear communication of results and the development of clinical management guidelines. *The Bethesda System for Reporting Cervical Cytology* was developed in 1988 and last revised in 2014 [20]. The recommended report includes an adequacy statement followed by the diagnosis. The general descriptor "epithelial cell abnormality," which encompasses all squamous and glandular abnormalities, is followed by the specific diagnosis.

The sensitivity of cytology for detecting preinvasive and invasive squamous and glandular lesions is difficult to establish and estimates range widely [48]. Discrepancies between cervical cytology and biopsies have been attributed to sampling errors. Routinely providing the patient's recent cervical cytology report to the surgical pathologist at the time the biopsy is examined results in improved sensitivity [49].

As with surgical biopsies, interobserver variability in the interpretation of cervical cytology has been documented, ASCUS representing the greatest source of variability [27].

Routine cervical sampling results in exfoliation of the superficial layers of the cervical epithelium. In women of reproductive age, the predominant cells are mature superficial and intermediate squamous cells, in variable proportions, depending on the phase of the menstrual cycle. Mature squamous cells have abundant pink or green cytoplasm and small dark nuclei on the Papanicolaou stain (Fig. 5.2a, b). Parabasal cells, immature cells located below the intermediate cells in the normal squamous epithelium, are usually not abundant in premenopausal women. Parabasal cells have less abundant and denser cytoplasm and, hence, higher nuclear-to-cytoplasmic ratio, than intermediate cells (Fig. 5.2c). Parabasal cells are the predominant cell in the atrophic smears of postmenopausal and postpartum women. Endocervical glandular and squamous metaplastic cells are part of an optimal cervical sample.

Approximately 90% of cervical cytology specimens are interpreted as negative for intraepithelial lesion or malignancy [50].

The Bethesda System terminology for reporting of noninvasive cervical squamous neoplasia is two-tiered: low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL) [20].

The diagnosis of SIL in cervical cytology rests on the identification of morphological changes in the nuclei of the squamous epithelial cells and alterations in the

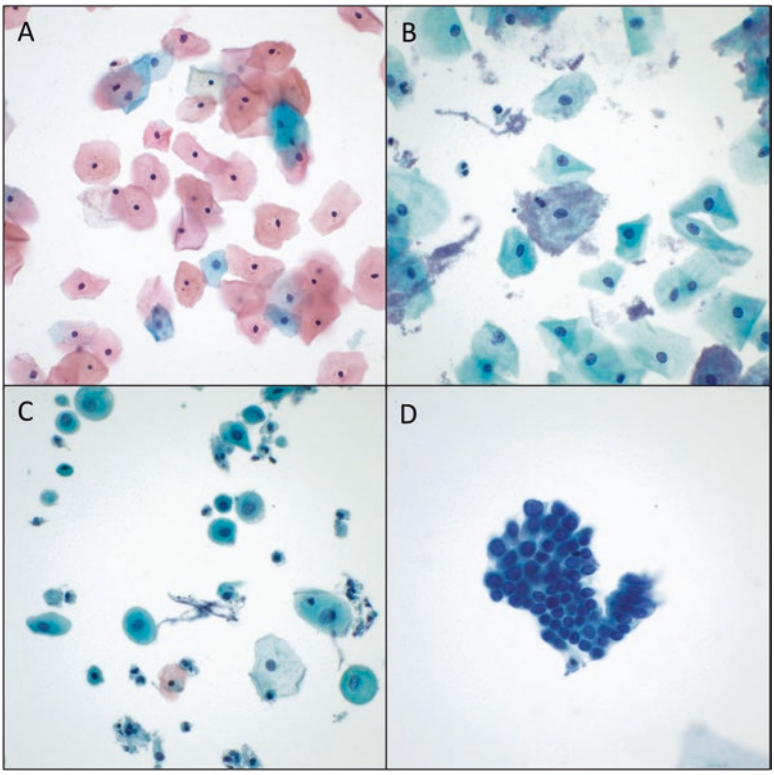

Fig. 5.2 Normal cells in Pap (ThinPrep 600X, Papanicolaou stain). (**a**): Predominance of superficial cells with abundant polygonal pink and light blue cytoplasm and pyknotic nuclei. (**b**): Predominance of intermediate cells with abundant polygonal pink and light blue cytoplasm and larger nuclei. This case also shows shift in flora suggestive of bacterial vaginosis. (**c**): Predominance of parabasal cells in an atrophic smear. The cells have smaller denser cytoplasm compared to superficial and intermediate cells. (**d**): Endocervical cells forming a honeycomb structure. The linear arrangement of the cells toward the right side shows the columnar cell shape with basal nuclei

nuclear-to-cytoplasmic ratio. Normal intermediate cells are used as comparison. Changes of SIL include nuclear enlargement (greater than three times the size of a normal intermediate cell nucleus), irregular nuclear contours, and chromatin clumping or hyperchromasia. Grading of SIL relies on evaluation of the amount of cytoplasm in the dysplastic cells.

Cells of LSIL have abundant cytoplasm, comparable to the amount of cytoplasm in superficial or intermediate cells. The dysplastic nuclei of LSIL may be surrounded by a clear halo, a morphological change known as koilocytosis, which reflects HPV cytopathic effect (Fig. 5.3b).

The cells of HSIL have scant cytoplasm and a greater degree of nuclear abnormalities, such as nuclear irregularities and hyperchromasia, than those of LSIL (Fig. 5.3c).

Atypical squamous cells of undetermined significance (ASCUS) is a term used in the Bethesda classification when some but not all the morphological features of dysplasia are met or when diagnostic changes of dysplasia are present only in a few cells (Fig. 5.3a). Atypical squamous cells of undetermined significance cannot exclude high-grade lesion (ASCUS-H) is the term used when these incompletely developed nuclear changes are seen in cells with scant cytoplasm. The ASC-H category implies a higher risk for HSIL in follow-up biopsy than ASCUS (50% vs 17%) [51].

SIL must be distinguished from benign mimics. Atrophic smears in postmenopausal and postpartum women show tight groups of cells with dark nuclei and high nuclear-to-cytoplasmic ratio, which may resemble HSIL or ASCUS. Repeat sampling after topical intravaginal estrogen cream causes cellular maturation and may be helpful in those cases [52].

Other mimics of SIL on cervical cytology include cellular changes due to radiation therapy and reactive changes associated with inflammation or intrauterine devices (IUD).

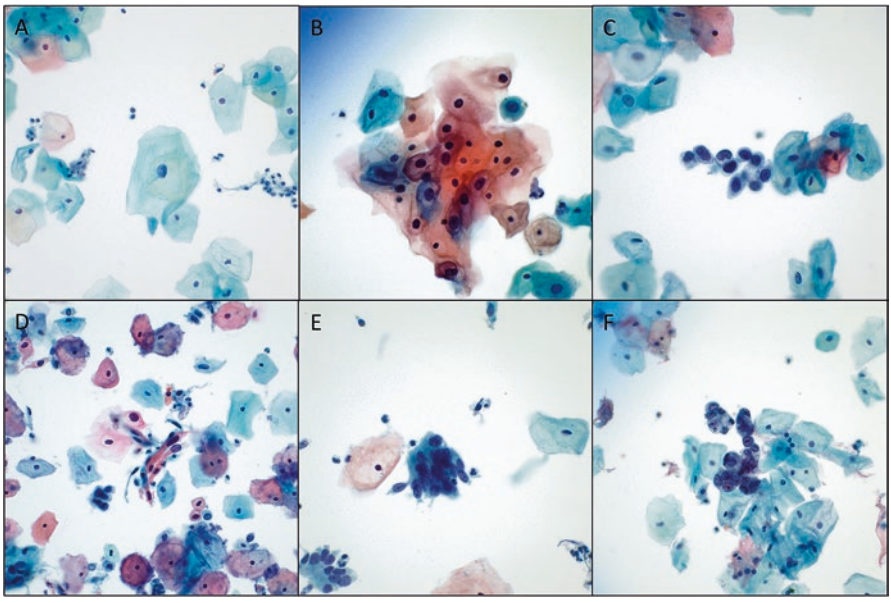

Fig. 5.3 Abnormal Pap (ThinPrep 600X, Papanicolaou stain). (**a**): ASCUS case with a mature cell showing nuclear enlargement less than three times the size of intermediate cells nuclei. (**b**): LSIL with koilocytes. Notice the nuclear abnormalities associated with a perinuclear halo. (**c**): HSIL cells with high nuclear-to-cytoplasmic ratio and marked nuclear abnormalities. (**d**): Invasive squamous cell carcinoma with orangeophilic "fiber cells." (**e**): Endocervical adenocarcinoma in situ with a "picket fence" group of columnar cells showing pseudostratification and "feathering" on the left side. (**f**): Invasive endometrial adenocarcinoma with tight groups of cells with vacuolated cytoplasm and prominent nucleoli

Invasive Squamous Carcinoma

Surgical Pathology

Squamous cell carcinoma accounts for approximately 75% of invasive cervical carcinomas [53]. It is caused by persistent HR HPV infections [54], most commonly with HPV 16/18 [55].

Advanced tumors form an exophytic or ulcerated mass in the cervix. Early cancers may not be detectable grossly.

Squamous cell carcinomas (SCC) may show a variety of histological patterns. Keratinizing SCC is characterized by the formation of keratin pearls, in which tumor cells surround central areas of extracellular keratin. Cells are polygonal with relatively abundant eosinophilic cytoplasm. Additional features include intercellular bridges and individual cell keratinization (Fig. 5.1f).

Nonkeratinizing squamous cell carcinomas are less differentiated than keratinizing carcinomas and lack keratin pearls. Nests or cords of polygonal tumor cells invade the underlying stroma, inducing a desmoplastic or inflammatory reaction.

Squamous cell carcinomas that display a warty or papillary architecture include warty/condylomatous, papillary, and squamo-transitional carcinoma [56–58]. As these tumors are exophytic, their invasive nature may not be apparent in superficial biopsies. Verrucous carcinoma, an extremely well-differentiated variant of SCC seen more commonly in the vulva, is very rare in the cervix [59]. The thickened, hyperkeratotic squamous epithelium in these exophytic tumors shows minimal cytologic atypia, such that diagnosis is not possible in superficial biopsies [59].

Basaloid SCC is an aggressive, high-grade variant of SCC composed of small hyperchromatic cells with a high nuclear-to-cytoplasmic ratio surrounding areas of comedo-type necrosis [1, 60].

Lymphoepithelioma-like carcinoma is a rare variant of SCC that resembles the tumors more commonly seen in the nasopharynx. While the nasopharyngeal tumors are associated with Epstein-Barr virus (EBV) infection, EBV has not been demonstrated in most of the cervical tumors [61–63], which instead appear to be associated with HPV [62, 63]. Small groups of tumor cells with ill-defined cell borders are obscured by an intense lymphoplasmacytic infiltrate. Immunohistochemical stains for keratin or the squamous cell markers p63 or p40 highlight the epithelial cells amidst the inflammatory cells.

SCCs are graded as well, moderately, or poorly differentiated, according to how much they resemble squamous epithelium. In general, though various grading systems have been used [64], less keratinization, increased cellular pleomorphism, and mitotic activity correlate with higher grades. While routinely reported, histologic grade has not been shown to predict behavior in cervical SCC [64].

The TNM (tumor, node, and metastasis) staging system (developed by the American Joint Committee on Cancer (AJCC) in collaboration with the Union for International Cancer Control (UICC)) and the Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) staging system are the most widely used staging systems of cervical cancer (AJCC) [65]. Pathological staging of invasive cervical carcinoma encompasses tumor size, depth of invasion, and extrauterine

extension. Depth of invasion is measured from the base of the epithelium where the invasion arises (surface or glandular). Tangential sectioning, poorly oriented specimens, and a lack of noninvolved stromal/epithelial interface are some of the difficulties encountered in measuring depth of invasion.

Cytology

Invasive keratinizing SCC is characterized by cells with dense, orange (orangeophilic) cytoplasm and small pyknotic ("ink dot") nuclei or enlarged nuclei with prominent nucleoli. Cytoplasmic projections result in elongated "fiber" cells or "tadpoles" (Fig. 5.3d). Necrotic debris and degenerating blood (tumor diathesis) are seen in the background.

The cells of nonkeratinizing SCC resemble those seen in HSIL but, unlike HSIL, have prominent nucleoli and a background of tumor diathesis. The tumor diathesis may be difficult to appreciate, particularly in liquid-based preparations, and if scant or absent, the specimen may be interpreted as HSIL rather than invasive carcinoma [66, 67].

The features of other less common variants of SCC have not been well described in cytology specimens [20, 68–72].

Glandular Lesions of the Cervix

Endocervical Adenocarcinoma In Situ

Surgical Pathology

The majority (~70–90%) of cervical adenocarcinomas are associated with HR HPV infection, particularly HPV 16 and 18 [73–75]. However, non-HPV-related subtypes of adenocarcinomas are increasingly being recognized [75–77] with important implications for screening and vaccination efforts.

Precursor Lesions Adenocarcinoma in situ (AIS) is the precursor lesion of the usual type of invasive adenocarcinoma. In AIS, neoplastic cells with elongated, hyperchromatic nuclei and high nuclear-to-cytoplasmic ratio replace the surface epithelium and the preexisting endocervical glands (Fig. 5.1d). An increased mitotic rate and apoptotic bodies are characteristic [78]. The cells of AIS of the cervix may resemble endometrial cells (endometrioid type of AIS). Goblet cells characterize the unusual intestinal variant of AIS.

An unusual intraepithelial lesion, stratified mucin-producing intraepithelial lesion (SMILE), is considered a variant of AIS. The stratified epithelium resembles HSIL but has admixed intracytoplasmic mucin vacuoles [79]. A distinctive variant of invasive adenocarcinoma associated with this lesion has recently been described [80].

The precursor lesions of non-HPV-related adenocarcinomas have not been well-defined. It is thought that lobular endocervical glandular hyperplasia or atypical

lobular endocervical glandular hyperplasia, in which there is a proliferation of glands with gastric-type epithelium, may be the precursor of the gastric-type endocervical adenocarcinoma [81, 82], the most common of the non-HPV-related adenocarcinomas. Recently, a type of AIS with gastric and, in some cases, intestinal differentiation has been described ("gastric-type AIS") as another possible precursor to gastric-type adenocarcinoma [83].

Mimics of AIS include tubal and tubulo-endometrioid metaplasia. Identification of ciliated cells, rare in AIS, aids in this distinction. Cervical endometriosis may be confused with adenocarcinoma if the endometrial-type stroma is not recognized. Reactive endocervical glands have prominent nucleoli, preservation of N/C ratio, and no or infrequent mitosis or apoptotic cells. Pregnancy-related changes, including Arias-Stella reaction, may also raise concern for adenocarcinoma. Microglandular hyperplasia, a benign proliferation of glands, lacks cellular atypia or mitotic activity.

The same biomarkers that differentiate SIL from benign mimics may be useful in the distinction of HPV-related adenocarcinoma from benign mimics. p16 shows diffuse staining in HPV-related AIS and the usual type of invasive adenocarcinoma [84, 85] but may be negative in other subtypes of adenocarcinoma [83]. The proliferation marker Ki-67 is increased in AIS, whereas the anti-apoptotic marker Bcl-2 is usually completely or partially lost in AIS, as compared to normal epithelium [85].

Cytology

Normal endocervical cells in cytology preparations have small, round, basal nuclei with small nucleoli and apical mucinous cytoplasm (Fig. 5.2d). They may be dispersed as single cells or form one-dimensional clusters which resemble a picket fence or, if seen in cross section, a honeycomb.

Adenocarcinoma in situ (AIS), the precursor of the usual type of adenocarcinoma, is characterized by overlapping, three-dimensional groups of columnar cells with elongated nuclei at the periphery of the clusters (a characteristic feature called "feathering") [86]. The cells have hyperchromatic, elongated nuclei and high nuclear-to-cytoplasmic ratio (Fig. 5.3e). As in histological sections, apoptosis and mitoses, an indication of high cellular turnover, are also seen [87].

"Atypical glandular cells of undetermined significance (AGC)" is the term used when the morphological changes of AIS are incomplete or present only in a few cells. AGC is a poorly reproducible diagnosis [88]. On follow-up, 70–85% of cases with a diagnosis of AGC are benign [89, 90], and the histologic diagnoses include no significant histopathology, reactive changes, endometrial or endocervical polyps, tubal metaplasia, endometritis, and microglandular hyperplasia [90]. HSIL is more commonly diagnosed than cervical glandular neoplasia in patients with AGC on cytology [89]. Five percentage of patients with AGC have a diagnosis of carcinoma, most commonly ovarian or endometrial adenocarcinoma [90].

AGC may be further characterized into atypical endocervical or atypical endometrial cells, a distinction that is not always possible.

Invasive Endocervical Adenocarcinoma

Surgical Pathology

Endocervical adenocarcinoma represents approximately 15% of cervical cancers in the United States [40].

Grossly, invasive cervical adenocarcinoma may appear as an exophytic or ulcerated cervical mass or diffuse thickening and induration of the endocervical canal. Small lesions may not be visible grossly.

Invasive adenocarcinoma may show a variety of morphologies. The most common type of endocervical adenocarcinoma, endocervical adenocarcinoma, usual type, is associated with HR HPV infection. The tumor cells have elongated, hyperchromatic nuclei and apical eosinophilic cytoplasm with variable amounts of intracytoplasmic mucin (Fig. 5.1e). As in AIS, mitosis and apoptotic bodies are frequent. Evidence of stromal invasion is seen in increased architectural complexity with papillary formation, cribriform spaces, and irregularly shaped glands extending deeper into the cervical stroma than normal endocervical glands. A stromal fibrotic, edematous, or inflammatory reaction surrounds the neoplastic glands.

The endometrioid type of endocervical adenocarcinoma shows overlapping morphological features with the usual type of adenocarcinoma. It must be distinguished from adenocarcinoma originating in the endometrium. Immunohistochemistry is helpful in these cases with primary endocervical adenocarcinoma being positive for p16 and CEA and negative for estrogen and progesterone receptors and vimentin. The opposite staining pattern is seen in endometrial adenocarcinoma.

Unlike for SCC, histologic grading of adenocarcinoma has been shown to have prognostic value [91]. Grading of the usual type of adenocarcinoma is largely based on the proportion of solid vs glandular components. Grade 1 (well-differentiated) adenocarcinoma has 10% or less of solid growth, whereas grade 3 (poorly differentiated) has 50% or more of solid growth.

Mucinous adenocarcinoma, an unusual variant characterized by intracytoplasmic mucin, may be of the intestinal, signet ring cell, or gastric type. The intestinal [92] and signet ring cell [93] types are extremely rare primary cervical tumors that must be distinguished from secondary involvement of the cervix from a colorectal adenocarcinoma or metastasis from a breast or stomach primary, respectively.

The gastric type of mucinous adenocarcinoma is the most common type of non-HPV-related adenocarcinoma [94]. This tumor may be extremely well-differentiated, showing cytologically bland cells with abundant clear or pale eosinophilic cytoplasm lining haphazardly arranged, irregularly shaped glands that extend deeper into the stroma than normal endocervical glands (so-called adenoma malignum or minimal deviation adenocarcinoma). This pattern of invasion may be very subtle and difficult to diagnose. The presence of a clinically observed cervical mass and areas of overt cytological atypia or stromal desmoplasia serve to confirm the diagnosis. Despite the innocuous morphology, adenoma malignum has been associated with an aggressive clinical course [95]. While most cases appear to be sporadic [95], there is an association with Peutz-Jeghers syndrome, an autosomal dominant disorder resulting from germline mutations of the tumor suppressor gene *STK11* (*LKB1*)

[96]. *SKT11* somatic mutations have been identified in 55% of minimal deviation adenocarcinomas in patients without Peutz-Jeghers syndrome [97].

Other unusual endocervical adenocarcinomas include villoglandular adenocarcinoma, a well-differentiated adenocarcinoma with exophytic villous architecture seen in young women [98]. Clear cell adenocarcinoma, which may be sporadic or associated with intrauterine exposure to diethylstilbestrol (DES), shows similar morphology to the more common ovarian or endometrial tumors [99, 100]. Endocervical serous carcinoma, a rare primary endocervical tumor [101], is most commonly seen as a result of spread from an adnexal or endometrial primary. Mesonephric adenocarcinoma is an extremely rare cervical carcinoma that arises from the mesonephric remnants on the lateral cervical wall and that must be distinguished from benign mesonephric hyperplasia [102].

Cytology

Subclassification of the various types of cervical adenocarcinoma may not be possible on cytology specimens.

The usual type of invasive endocervical adenocarcinoma presents as large overlapping sheets of tumor cells with round nuclei and prominent nucleoli. The cytoplasm is usually abundant but ill-defined, light blue, and translucent. Background necrosis may be present [103].

The endometrioid type of adenocarcinoma may be morphologically indistinguishable from the endometrial counterpart. The cells are either isolated or disposed in tight three-dimensional groups of cells with round borders (Fig. 5.3f). The nuclei, similar in size to intermediate squamous cells, have prominent nucleoli. The presence of cytoplasmic vacuoles sometimes containing phagocytized neutrophils ("bags of polyps") is characteristic. Macrophages are commonly seen in the background along with tumor diathesis.

The mucinous type of endocervical adenocarcinoma may be very difficult to recognize in cytology specimens, particularly when well-differentiated. Predominance or abundance of endocervical cells and mild cytological atypia may be clues to the diagnosis [103–105]. Goblet cells, indicative of intestinal differentiation, may be seen.

Villoglandular carcinoma may be deceptively bland-looking in cytology specimens, resulting in its misdiagnosis as reactive glandular lesions [106].

Clear cell carcinoma has cells with pale nuclei, nucleoli, and foamy or granular cytoplasm, indistinguishable from the more common ovarian or endometrial clear cell carcinomas [107].

Unusual Cervical Neoplasms

Neuroendocrine tumors are rare in the cervix and are associated with HR HPV infection [108]. The WHO terminology and criteria for cervical neuroendocrine tumors have been adopted form the more common tumors of the gastrointestinal tract and pancreas [1, 109]. Low-grade neuroendocrine tumors are extremely rare

and include grade 1 and 2 neuroendocrine tumors (carcinoid and atypical carcinoids in older nomenclature). Grade 1 tumors show nests, cords, or ribbons of cells with speckled, "salt and pepper" chromatin. Grade 2 tumors differ from grade 1 tumors in displaying an increased mitotic rate as well as focal areas of necrosis.

High-grade neuroendocrine carcinomas are highly aggressive tumors which are most commonly of the small cell type, similar to small cell carcinoma of the lung. Small cell carcinoma is characterized by small cells, with scant cytoplasm, nuclear molding, and numerous mitoses. Large cell neuroendocrine carcinoma, a high-grade tumor with neuroendocrine morphology, expresses the neuroendocrine markers chromogranin, synaptophysin, and/or CD56 by immunohistochemistry [110].

Adenosquamous carcinoma shows malignant glandular and squamous components [1]. Glassy cell carcinoma, so called because of the abundant eosinophilic ground glass cytoplasm of the tumor cells, is thought to represent the undifferentiated form of adenosquamous carcinoma [111, 112].

Other rare miscellaneous tumors of the cervix are listed in Table 5.1 and include adenoid basal carcinoma [113], adenoid cystic carcinoma [114], undifferentiated carcinoma [1], carcinosarcoma (malignant mixed Müllerian tumor) [115, 116], malignant melanoma [117, 118], yolk sac tumor [119], and lymphoma [120].

Cytology

Definite diagnosis of uncommon tumors of the cervix may not be possible on cytologic specimens. Small cell carcinoma of the cervix, a high-grade neuroendocrine carcinoma, shows similar morphologic features as small cell carcinoma in other sites, such as the lung. The cells are small with scant cytoplasm, a speckled ("salt and pepper") chromatin pattern, and inconspicuous nucleoli. Nuclear molding, prominent crushed artifact, necrosis, apoptotic bodies, and mitosis are characteristic of this tumor [121].

Large cell neuroendocrine carcinoma cytology may show abortive rosettes and prominent eosinophilic nucleoli with abundant cytoplasm as well as "naked" nuclei devoid of cytoplasm [122]. Adenosquamous carcinoma shows recognizable squamous and glandular malignant morphology.

Cervical cytology may detect metastasis, most commonly from elsewhere in the gynecological tract [123]. Tumors, such as mesenchymal tumors which grow under intact cervical epithelium, may not yield diagnostic material on cytology specimens [1].

References

1. Kurman RJ, Carcangiu ML, Herrington CS, Young RH, editors. WHO classification of tumours of female reproductive organs. 4th ed. Lyon: IARC; 2014.
2. Haghshenas MR, Mousavi T, Kheradmand M, Afshari M, Moosazadeh M. Efficacy of human papillomavirus L1 protein vaccines (Cervarix and Gardasil) in reducing the risk of cervical intraepithelial neoplasia: a meta-analysis. Int J Prev Med. 2017;8:44.

3. Krishnamurti U, Unger ER. Pathobiology of human papillomaviruses in human immunodeficiency virus – infected persons. Semin Diagn Pathol. 2017;34(4):364–70.
4. Thomison J, Thomas LK, Shroyer KR. Human papillomavirus: molecular and cytologic/histologic aspects related to cervical intraepithelial neoplasia and carcinoma. Hum Pathol. 2008;39(2):154–66.
5. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol. 2017;32:16–24.
6. Lee L-Y, Garland SM. Human papillomavirus vaccination: the population impact. F1000Res. 2017;6:866.
7. Doorbar J. Host control of human papillomavirus infection and disease. Best Pract Res Clin Obstet Gynaecol. 2017;47:27–41.
8. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet. 2007;370(9590):890–907.
9. Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30(Suppl 5):F55–70.
10. Mirkovic J, Howitt BE, Roncarati P, Demoulin S, Suarez-Carmona M, Hubert P, et al. Carcinogenic HPV infection in the cervical squamo-columnar junction. J Pathol. 2015;236(3):265–71.
11. Burd EM. Human papillomavirus and cervical cancer. Clin Microbiol Rev. 2003;16(1):1–17.
12. Huh WK, Ault KA, Chelmow D, Davey DD, Goulart RA, Garcia FAR, et al. Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. Obstet Gynecol. 2015;125(2):330–7.
13. Committee on Practice Bulletins—Gynecology. Practice Bulletin No. 168: cervical cancer screening and prevention. Obstet Gynecol. 2016;128(4):e111–30.
14. Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, Scott DR, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst. 2005;97(14):1072–9.
15. Schiffman M, Burk RD, Boyle S, Raine-Bennett T, Katki HA, Gage JC, et al. A study of genotyping for management of human papillomavirus-positive, cytology-negative cervical screening results. J Clin Microbiol. 2015;53(1):52–9.
16. Arbyn M, Snijders PJF, Meijer CJLM, Berkhof J, Cuschieri K, Kocjan BJ, et al. Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening? Clin Microbiol Infect. 2015;21(9):817–26.
17. Zhao C, Moriarty AT, Ghofrani M, Husain M, Tambouret RH, Laucirica R, et al. Human papillomavirus testing and reporting rates in 2012: results of a College of American Pathologists national survey. Arch Pathol Lab Med. 2014;139(6):757–61.
18. de Thurah L, Bonde J, Lam JUH, Rebolj M. Concordant testing results between various human papillomavirus assays in primary cervical cancer screening: systematic review. Clin Microbiol Infect. 2017;24:29–36.
19. Mills AM, Dirks DC, Poulter MD, Mills SE, Stoler MH. HR-HPV E6/E7 mRNA in situ hybridization: validation against PCR, DNA in situ hybridization, and p16 immunohistochemistry in 102 samples of cervical, vulvar, anal, and head and neck neoplasia. Am J Surg Pathol. 2017;41(5):607–15.
20. Nayar R, Wilbur DC, editors. The Bethesda system for reporting cervical cytology: definitions, criteria, and explanatory notes. 3rd ed. Cham: Springer; 2015.
21. Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, McCalmont T, Nayar R, Palefsky JM, Stoler MH, Wilkinson EJ. The lower anogenital squamous terminology standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. Arch Pathol Lab Med. 2012;136(10):1266–97.
22. Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, Solomon D, Wentzensen N, Lawson HW. 2012 updated consensus guidelines for the manage-

ment of abnormal cervical cancer screening tests and cancer precursors. Obstet Gynecol. 2013;121(4):829–46.
23. Doorbar J. Model systems of human papillomavirus-associated disease. J Pathol. 2016;238(2):166–79.
24. McCluggage WG, Walsh MY, Thornton CM, Hamilton PW, Caughley LM, Bharucha H. Inter- and intra-observer variation in the histopathological reporting of cervical squamous in intraepithelial lesion using a modified Bethesda grading system. BJOG. 1998;105(2):206–10.
25. Carreon JD, Sherman ME, Guillén D, Solomon D, Herrero R, Jerónimo J, Wacholder S, Rodríguez AC, Morales J, Hutchinson M, Burk RD. CIN2 is a much less reproducible and less valid diagnosis than CIN3: results from a histological review of population-based cervical samples. Int J Gynecol Pathol. 2007;26(4):441–6.
26. Castle PE, Stoler MH, Solomon D, Schiffmanx M, for the ALTS Group. The relationship of community biopsy-diagnosed cervical intraepithelial neoplasia grade 2 to the quality control pathology-reviewed diagnoses: an ALTS report. Am J Clin Pathol. 2007;127(5):805–15.
27. Stoler MH, Schiffman M, for the Atypical Squamous Cells of Undetermined Significance-Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS) Group. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL triage study. JAMA. 2001;285(11):1500–5.
28. Galgano MT, Castle PE, Atkins KA, Brix WK, Nassau SR, Stoler MH. Using biomarkers as objective standards in the diagnosis of cervical biopsies. Am J Surg Pathol. 2010;34(8):1077–87.
29. Group TA. A randomized trial on the management of low-grade squamous intraepithelial lesion cytology interpretations. Am J Obstet Gynecol. 2003;188(6):1393–400.
30. Van Baars R, Griffin H, Wu Z, Soneji YJ, Van de Sandt M, Arora R, et al. Investigating diagnostic problems of CIN 1 and 2 associated with high-risk HPV by combining the novel molecular biomarker PanHPV E4 with P16(ink4a). Am J Surg Pathol. 2015;39(11):1518–28.
31. Tsoumpou I, Arbyn M, Kyrgiou M, Wentzensen N, Koliopoulos G, Martin-Hirsch P, et al. p16(INK4a) immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis. Cancer Treat Rev. 2009;35(3):210–20.
32. Conesa-Zamora P, Domenech-Peris A, Orantes-Casado FJ, Ortiz-Reina S, Sahuquillo-Frias L, Acosta-Ortega J, et al. Effect of human papillomavirus on cell cycle-related proteins p16, Ki-67, cyclin D1, p53, and ProEx C in precursor lesions of cervical carcinoma: a tissue microarray study. Am J Clin Pathol. 2009;132(3):378–90.
33. Bergeron C, Ordi J, Schmidt D, Trunk MJ, Keller T, Ridder R. Conjunctive p16INK4a testing significantly increases accuracy in diagnosing high-grade cervical intraepithelial neoplasia. Am J Clin Pathol. 2010;133(3):395–406.
34. Pinto AP, Schlecht NF, Woo TYC, Crum CP, Cibas ES. Biomarker (ProEx C, p16(INK4A), and MiB-1) distinction of high-grade squamous intraepithelial lesion from its mimics. Mod Pathol. 2008;21(9):1067–74.
35. Dijkstra MG, Heideman DAM, de Roy SC, Rozendaal L, Berkhof J, Van Krimpen K, et al. p16(INK4a) immunostaining as an alternative to histology review for reliable grading of cervical intraepithelial lesions. J Clin Pathol. 2010;63(11):972–7.
36. Pinto AP, Crum CP, Hirsch MS. Molecular markers of early cervical neoplasia. Diagn Histopathol (Oxf). 2010;16(10):445–54.
37. Maniar KP, Sanchez B, Paintal A, Gursel DB, Nayar R. Role of the biomarker p16 in downgrading -IN 2 diagnoses and predicting higher-grade lesions. Am J Surg Pathol. 2015;39(12):1708–18.
38. del Pino M, Garcia S, Fuste V, Alonso I, Fuste P, Torne A, et al. Value of p16(INK4a) as a marker of progression/regression in cervical intraepithelial neoplasia grade 1. Am J Obstet Gynecol. 2009;201(5):488.e1-7.
39. Quint KD, de Koning MNC, Quint WGV, Pirog EC. Progression of cervical low grade squamous intraepithelial lesions: in search of prognostic biomarkers. Eur J Obstet Gynecol Reprod Biol. 2013;170(2):501–6.

40. Wang SS, Trunk M, Schiffman M, Herrero R, Sherman ME, Burk RD, Hildesheim A, Bratti MC, Wright T, Rodriguez AC, Chen S. Validation of p16INK4a as a marker of oncogenic human papillomavirus infection in cervical biopsies from a population-based cohort in Costa Rica. Cancer Epidemiol Biomarkers Prev. 2004;13(8):1355–60.
41. Sagasta A, Castillo P, Saco A, Torne A, Esteve R, Marimon L, et al. p16 staining has limited value in predicting the outcome of histological low-grade squamous intraepithelial lesions of the cervix. Mod Pathol. 2016;29(1):51–9.
42. Moyer VA. Screening for cervical cancer: US Preventive Services Task Force recommendation statement. Ann Intern Med. 2012;156(12):880–91.
43. Saslow D, Solomon D, Lawson HW, Killackey M, Kulasingam SL, Cain J, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. CA Cancer J Clin. 2012;62(3):147–72.
44. Bernstein SJ, Sanchez-Ramos L, Ndubisi B. Liquid-based cervical cytologic smear study and conventional Papanicolaou smears: a metaanalysis of prospective studies comparing cytologic diagnosis and sample adequacy. Am J Obstet Gynecol. 2001;185(2):308–17.
45. Stein SR. ThinPrep versus the conventional Papanicolaou test: a review of specimen adequacy, sensitivity, and cost-effectiveness. Prim Care Update OB Gyns. 2003;10(6):310–3.
46. Schiffman M, Solomon D. Screening and prevention methods for cervical cancer. JAMA. 2009;302(16):1809–10.
47. https://www.cancer.org/cancer/cervical-cancer/prevention-and-early-detection/pap-test.html. Accessed 20 Aug 2017.
48. Cibas ES, Ducatman BS. Cervical and vaginal cytology. In: Cytology. Diagnostic principles and clinical correlates. 4th ed. Philadelphia: Saunders; 2014. p. 7–8.
49. Jones BA, Novis DA. Cervical biopsy-cytology correlation. A College of American Pathologists Q-Probes study of 22,439 correlations in 348 laboratories. Arch Pathol Lab Med. 1996;120:523–31.
50. Eversole GM, Moriarty AT, Schwartz MR, Clayton AC, Souers R, Fatheree LA, et al. Practices of participants in the College of American Pathologists interlaboratory comparison program in cervicovaginal cytology, 2006. Arch Pathol Lab Med. 2010;134(3):331–5.
51. Sherman ME, Castle PE, Solomon D. Cervical cytology of atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion (ASC-H): characteristics and histologic outcomes. Cancer. 2006;108(5):298–305.
52. Thomas C, Wright J, Cox JT, Massad LS, Twiggs LB, Wilkinson EJ. Conference for the 2001 A-SC. 2001 Consensus Guidelines for the management of women with cervical cytological abnormalities. JAMA. 2002;287(16):2120–9.
53. Vizcaino AP, Moreno V, Bosch FX, Munoz N, Barros-Dios XM, Borras J, et al. International trends in incidence of cervical cancer: II. Squamous-cell carcinoma. Int J Cancer. 2000;86(3):429–35.
54. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189(1):12–9.
55. De Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol. 2010;11(11):1048–56.
56. Brinck U, Jakob C, Bau O, Fuzesi L. Papillary squamous cell carcinoma of the uterine cervix: report of three cases and a review of its classification. Int J Gynecol Pathol. 2000;19(3):231–5.
57. Koenig C, Turnicky RP, Kankam CF, Tavassoli FA. Papillary squamotransitional cell carcinoma of the cervix: a report of 32 cases. Am J Surg Pathol. 1997;21(8):915–21.
58. Randall ME, Andersen WA, Mills SE, Kim JA. Papillary squamous cell carcinoma of the uterine cervix: a clinicopathologic study of nine cases. Int J Gynecol Pathol. 1986;5(1):1–10.
59. Zbroch T, Grzegorz Knapp P, Knapp PA. Verrucous carcinoma of the cervix – diagnostic and therapeutic difficulties with regards to HPV status. Case report. Eur J Gynaecol Oncol. 2005;26(2):227–30.

60. Grayson W, Cooper K. A reappraisal of "basaloid carcinoma" of the cervix, and the differential diagnosis of basaloid cervical neoplasms. Adv Anat Pathol. 2002;9(5):290–300.
61. Martorell MA, Julian JM, Calabuig C, Garcia-Garcia JA, Perez-Valles A. Lymphoepithelioma-like carcinoma of the uterine cervix. Arch Pathol Lab Med. 2002;126(12):1501–5.
62. Chao A, Tsai CN, Hsueh S, Lee LY, Chen TC, Huang SL, Chao FY, Lai CH. Does Epstein-Barr virus play a role in lymphoepithelioma-like carcinoma of the uterine cervix? Int J Gynecol Pathol. 2009;28(3):279–85.
63. Noel J, Lespagnard L, Fayt I, Verhest A, Dargent J. Evidence of human papilloma virus infection but lack of Epstein-Barr virus in lymphoepithelioma-like carcinoma of uterine cervix: report of two cases and review of the literature. Hum Pathol. 2001;32(1):135–8.
64. Zaino RJ, Ward S, Delgado G, Bundy B, Gore H, Fetter G, et al. Histopathologic predictors of the behavior of surgically treated stage IB squamous cell carcinoma of the cervix. A Gynecologic Oncology Group study. Cancer. 1992;69(7):1750–8.
65. Amin MB. Cervix uteri. In: AJCC cancer staging manual. 8th ed. New York: Springer; 2017. p. 649–59.
66. Rushing L, Cibas ES. The frequency of tumor diathesis in smears from women with squamous cell carcinoma of the cervix. Acta Cytol. 1997;41:781–5.
67. Levine PH, Elgert PA, Mittal K. False-positive squamous cell carcinoma in cervical smears: cytologic-histologic correlation in 19 cases. Diagn Cytopathol. 2003;28(1):23–7.
68. Bibbo M, Wilbur D. Comprehensive cytopathology. 4th ed. Philadelphia: Saunders; 2014. p. 161.
69. Joshi D, Shivkumar VB, Sharma SM, Gangane N. Cytomorphologic diagnosis of basaloid squamous cell carcinoma: a case report. Acta Cytol. 2009;53(1):89–92.
70. Frega A, Lukic A, Nobili F, Palazzo A, Iacovelli R, French D, et al. Verrucous carcinoma of the cervix: detection of carcinogenetic human papillomavirus types and their role during follow-up. Anticancer Res. 2007;27(6C):4491–4.
71. Martínez-Girón R, Martínez-Torre S, Mosquera-Martínez AJ. Basaloid squamous cell carcinoma of the uterine cervix: cytological and histological features. Diagn Cytopathol. 2015;43:993–5.
72. Ng W-K, Cheung LKN, Li ASM. Warty (condylomatous) carcinoma of the cervix. A review of 3 cases with emphasis on thin-layer cytology and molecular analysis for HPV. Acta Cytol. 2003;47(2):159–66.
73. Andersson S, Rylander E, Larsson B, Strand A, Silfversvard C, Wilander E. The role of human papillomavirus in cervical adenocarcinoma carcinogenesis. Eur J Cancer. 2001;37(2):246–50.
74. Iwasawa A, Nieminen P, Lehtinen M, Paavonen J. Human papillomavirus DNA in uterine cervix squamous cell carcinoma and adenocarcinoma detected by polymerase chain reaction. Cancer. 1996;77(11):2275–9.
75. An HJ, Kim KR, Kim IS, Kim DW, Park MH, Park IA, et al. Prevalence of human papillomavirus DNA in various histological subtypes of cervical adenocarcinoma: a population-based study. Mod Pathol. 2005;18(4):528–34.
76. Holl K, Nowakowski AM, Powell N, McCluggage WG, Pirog EC, Collas De Souza S, et al. Human papillomavirus prevalence and type-distribution in cervical glandular neoplasias: results from a European multinational epidemiological study. Int J Cancer. 2015;137(12):2858–68.
77. Pirog EC, Lloveras B, Molijn A, Tous S, Guimerà N, Alejo M, et al. HPV prevalence and genotypes in different histological subtypes of cervical adenocarcinoma, a worldwide analysis of 760 cases. Mod Pathol. 2014;27(12):1559.
78. Biscotti CV, Hart WR. Apoptotic bodies: a consistent morphologic feature of endocervical adenocarcinoma in situ. Am J Surg Pathol. 1998;22(4):434–9.
79. Park JJ, Sun D, Quade BJ, Flynn C, Sheets EE, Yang A, et al. Stratified mucin-producing intraepithelial lesions of the cervix: adenosquamous or columnar cell neoplasia? Am J Surg Pathol. 2000;24(10):1414–9.
80. Lastra RR, Park KJ, Schoolmeester JK. Invasive stratified mucin-producing carcinoma and stratified mucin-producing intraepithelial lesion (SMILE): 15 cases presenting a Spectrum of

cervical neoplasia with description of a distinctive variant of invasive adenocarcinoma. Am J Surg Pathol. 2016;40(2):262–9.
81. Mikami Y, Kiyokawa T, Hata S, Fujiwara K, Moriya T, Sasano H, et al. Gastrointestinal immunophenotype in adenocarcinomas of the uterine cervix and related glandular lesions: a possible link between lobular endocervical glandular hyperplasia/pyloric gland metaplasia and "adenoma malignum". Mod Pathol. 2004;17(8):962–72.
82. Kawauchi S, Kusuda T, Liu X-P, Suehiro Y, Kaku T, Mikami Y, et al. Is lobular endocervical glandular hyperplasia a cancerous precursor of minimal deviation adenocarcinoma?: a comparative molecular-genetic and immunohistochemical study. Am J Surg Pathol. 2008;32(12):1807–15.
83. Talia KL, Stewart CJ, Howitt BE, Nucci MR, McCluggage WG. HPV-negative gastric type adenocarcinoma in situ of the cervix: a Spectrum of rare lesions exhibiting gastric and intestinal differentiation. Am J Surg Pathol. 2017;41(8):1023–33.
84. Negri G, Egarter-Vigl E, Kasal A, Romano F, Haitel A, Mian C. p16INK4a is a useful marker for the diagnosis of adenocarcinoma of the cervix uteri and its precursors: an immunohistochemical study with immunocytochemical correlations. Am J Surg Pathol. 2003;27(2):187–93.
85. Cameron RI, Maxwell P, Jenkins D, McCluggage WG. Immunohistochemical staining with MIB1, bcl2 and p16 assists in the distinction of cervical glandular intraepithelial neoplasia from tubo-endometrial metaplasia, endometriosis and microglandular hyperplasia. Histopathology. 2002;41(4):313–21.
86. Biscotti CV, Gero MA, Toddy SM, Fischler DF, Easley KA. Endocervical adenocarcinoma in situ: an analysis of cellular features. Diagn Cytopathol. 1997;17(5):326–32.
87. Lee KR, Manna EA, Jones MA. Comparative cytologic features of adenocarcinoma in situ of the uterine cervix. Acta Cytol. 1991;35:117–26.
88. Lee KR, Darragh TM, Joste NE, Krane JF, Sherman ME, Hurley LB, et al. Atypical glandular cells of undetermined significance (AGUS) Interobserver reproducibility in cervical smears and corresponding thin-layer preparations. Am J Clin Pathol. 2002;117(1):96–102.
89. Schnatz PF, Guile M, O'sullivan DM, Sorosky JI. Clinical significance of atypical glandular cells on cervical cytology. Obstet Gynecol. 2006;107(3):701–8.
90. Zhao C, Florea A, Onisko A, Austin RM. Histologic follow-up results in 662 patients with Pap test findings of atypical glandular cells: results from a large academic womens hospital laboratory employing sensitive screening methods. Gynecol Oncol. 2009;114(3):383–9.
91. Baalbergen A, Ewing-Graham PC, Hop WCJ, Struijk P, Helmerhorst TJM. Prognostic factors in adenocarcinoma of the uterine cervix. Gynecol Oncol. 2004;92(1):262–7.
92. McCluggage WG, Shah R, Connolly LE, McBride HA. Intestinal-type cervical adenocarcinoma in situ and adenocarcinoma exhibit a partial enteric immunophenotype with consistent expression of CDX2. Int J Gynecol Pathol. 2008;27(1):92–100.
93. Balci S, Saglam A, Usubutun A. Primary signet-ring cell carcinoma of the cervix: case report and review of the literature. Int J Gynecol Pathol. 2010;29(2):181–4.
94. McCluggage WG. Recent developments in non-HPV-related adenocarcinomas of the lower female genital tract and their precursors. Adv Anat Pathol. 2016;23(1):58–69.
95. Gilks CB, Young RH, Aguirre P, DeLellis RA, Scully RE. Adenoma malignum (minimal deviation adenocarcinoma) of the uterine cervix. A clinicopathological and immunohistochemical analysis of 26 cases. Am J Surg Pathol. 1989;13(9):717–29.
96. Jenne DE, Reimann H, Nezu J, Friedel W, Loff S, Jeschke R, et al. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. Nat Genet. 1998;18(1):38–43.
97. Kuragaki C, Enomoto T, Ueno Y, Sun H, Fujita M, Nakashima R, et al. Mutations in the STK11 gene characterize minimal deviation adenocarcinoma of the uterine cervix. Lab Investig. 2003;83(1):35–45.
98. Jones MW, Silverberg SG, Kurman RJ. Well-differentiated villoglandular adenocarcinoma of the uterine cervix: a clinicopathological study of 24 cases. Int J Gynecol Pathol. 1993;12(1):1–7.

99. Hanselaar A, Van Loosbroek M, Schuurbiers O, Helmerhorst T, Bulten J, Bernhelm J. Clear cell adenocarcinoma of the vagina and cervix. An update of the Central Netherlands registry showing twin age incidence peaks. Cancer. 1997;79(11):2229–36.
100. Kaminski PF, Maier RC. Clear cell adenocarcinoma of the cervix unrelated to diethylstilbestrol exposure. Obstet Gynecol. 1983;62(6):720–7.
101. Zhou C, Gilks CB, Hayes M, Clement PB. Papillary serous carcinoma of the uterine cervix: a clinicopathologic study of 17 cases. Am J Surg Pathol. 1998;22(1):113–20.
102. Ferry JA, Scully RE. Mesonephric remnants, hyperplasia, and neoplasia in the uterine cervix. A study of 49 cases. Am J Surg Pathol. 1990;14(12):1100–11.
103. Ayer B, Pacey F, Greenberg M. The cytologic diagnosis of adenocarcinoma in situ of the cervix uteri and related lesions. II. Microinvasive adenocarcinoma. Acta Cytol. 1988;32(3):318–24.
104. Granter SR, Lee KL. Cytologic findings in minimal deviation adenocarcinoma (adenoma malignum) of the cervix: a report of seven cases. Am J Clin Pathol. 1996;105:327–33.
105. Khalbuss W, Monaco S, Pantanowitz L. Cytomorphology of unusual primary tumors in the Pap test. CytoJournal. 2013;10:17.
106. Choi Y, Kim H, Choi H, Hwang D, Choe G, Chung J-H, et al. Liquid-based cytology of villoglandular adenocarcinoma of the cervix: a report of 3 cases. Korean J Pathol. 2012;46(2):215–20.
107. Hanselaar AGJM, Boss EA, Massuger LFAG, Bernheim JL. Cytologic examination to detect clear cell adenocarcinoma of the vagina or cervix. Gynecol Oncol. 1999;75:338–44.
108. Mannion C, Park WS, Man YG, Zhuang Z, Albores-Saavedra J, Tavassoli FA. Endocrine tumors of the cervix: morphologic assessment, expression of human papillomavirus and evaluation for loss of heterozygosity on 1p,3p, 11q, and 17p. Cancer. 1998;83(7):1391–400.
109. Bosman FT, Carneiro F, Hruban RH, Theise ND, editors. WHO classification of tumours of the digestive system. 4th ed. Lyon: IARC; 2014.
110. McCusker ME, Cote TR, Clegg LX, Tavassoli FJ. Endocrine tumors of the uterine cervix: incidence, demographics, and survival with comparison to squamous cell carcinoma. Gynecol Oncol. 2003;88(3):333–9.
111. Zaino RJ, Nahhas WA, Mortel R. Glassy cell carcinoma of the uterine cervix. An ultrastructural study and review. Arch Pathol Lab Med. 1982;106(5):250–4.
112. Guitarte C, Alagkiozidis I, Mize B, Stevens E, Salame G, Lee Y-C. Glassy cell carcinoma of the cervix: a systematic review and meta-analysis. Gynecol Oncol. 2014;133(2):186–91.
113. Chen T-D, Chuang H-C, Lee L. Adenoid basal carcinoma of the uterine cervix: clinicopathologic features of 12 cases with reference to CD117 expression. Int J Gynecol Pathol. 2012;31(1):25–32.
114. Ferry JA, Scully RE. "Adenoid cystic" carcinoma and adenoid basal carcinoma of the uterine cervix: a study of 28 cases. J Surg Pathol. 1988;12(2):134–44.
115. Abell MR, Ramirez GJA. Sarcomas and carcinosarcomas of the uterine cervix. Cancer. 1973;31(5):1176–92.
116. Kim M, Lee C, Choi H, Ko J-K, Kang G, Chun K. Carcinosarcoma of the uterine cervix arising from Müllerian ducts. Obstet Gynecol Sci. 2015;58(3):251.
117. Singh N, Tripathi R, Mala YM. Primary malignant melanoma of uterine cervix with probable origin from benign cervical melanosis. BMJ Case Rep [Internet] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3702895/pdf/bcr-2013-010042.pdf 2013 is repeated.
118. Myriokefalitaki E, Babbel B, Smith M, Ahmed AS. Primary malignant melanoma of uterine cervix FIGO IIa1: a case report with 40 months ongoing survival and literature review. Gynecol Oncol Case Rep. 2013;5:52–4.
119. Mardi K, Gupta N, Bindra R. Primary yolk sac tumor of cervix and vagina in an adult female: a rare case report. Indian J Cancer. 2011;48(4):515.
120. Bellevicine C, Zabatta A, Malapelle U, Vetrani A, Troncone G. Diffuse large B-cell extranodal lymphoma of the uterine cervix. Diagn Cytopathol. 2014;42(7):644–6.

121. Giorgadze T, Kanhere R, Pang C, Ganote C, Miller LE, Tabaczka P, Brown E, Husain M. Small cell carcinoma of the cervix in liquid-based Pap test: utilization of split-sample immunocytochemical and molecular analysis. Diagn Cytopathol. 2012;40:214–9.
122. Li S, Zhu H. Twelve cases of neuroendocrine carcinomas of the uterine cervix: cytology, histopathology and discussion of their histogenesis. Acta Cytol. 2013;57(1):54–60.
123. Sasagawa M, Nishino K, Honma S, Kodama S, Takahashi T. Origin of adenocarcinoma cells observed on cervical cytology. Acta Cytol. 2003;47:410–4.

Uterine Cervical Cancer in Women with HIV Infection

6

Linda Mileshkin, Evangeline Ponnusamy, and Catherine Louise Cherry

Squamous cell carcinoma of the uterine cervix is the commonest malignancy of the female genital tract. Management is multimodal, with surgery, chemotherapy, and radiotherapy all potentially having roles (either alone or in combination) depending on the stage of disease and patient factors. The prognosis of late stage disease is poor, but women diagnosed with precursor lesions (detectable with routine screening) and localized disease generally do well.

Infection with an oncogenic strain of HPV is thought to be a prerequisite for the development of cervical cancer, presenting opportunities for prevention. As with other HPV-associated malignancies, individuals who are HIV infected are at substantially increased risk for developing cervical cancer. It is an unfortunate reality that the highest burdens of both HIV and cervical cancer occur in low- and middle-income settings, where access to all of HPV vaccination, cervical screening programs, and treatment of established malignancies is often very limited.

Here we present an overview of current knowledge of the epidemiology, prevention, diagnosis, and management of squamous cell carcinoma of the uterine cervix, focusing on populations with HIV infection.

L. Mileshkin (✉) · E. Ponnusamy
Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia
e-mail: linda.mileshkin@petermac.org

C. L. Cherry
Department of Infectious Diseases, Monash University and Alfred Health, Melbourne, VIC, Australia

Burnet Institute, Melbourne, VIC, Australia

Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
e-mail: kate.cherry@burnet.edu.au

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_6

Epidemiology

Epidemiology of Cervical Cancer

Cervical cancer occurs worldwide [1]. The incidence of cervical cancer has decreased significantly in middle- and high-income countries over the last several decades. A similar trend, however, has not been seen in low-income countries where the incidence continues to be high. Nearly 80% of the global cervical cancer occurrence is confined to low- and middle-income countries, with East African countries, especially in the sub-Saharan region, having the highest incidence [2]. In these countries the mortality due to cervical cancer is about ten times higher than is seen in well-resourced settings, likely due to both inadequate screening and the lack of optimal treatment resources. In more developed areas of the world, it has been shown that screening for precursor lesions with conventional Pap smears is the main factor responsible for a major decrease in the incidence and mortality rates of cervical cancer in recent decades [3].

Cervical cancer deaths account for 7.5% of all female cancer deaths with an estimated 528,000 new cases and 266,000 deaths worldwide in 2012 (Fig. 6.1) [4]. A majority of these deaths occur in the less well-resourced regions. Mortality varies 18-fold between the different regions of the world, with rates ranging from less than 2 per 100,000 in Western Asia, Western Europe, and Australia/New Zealand to more than 20 per 100,000 in Melanesia (20.6) and Middle (22.2) and Eastern (27.6) Africa (Fig. 6.2) [4].

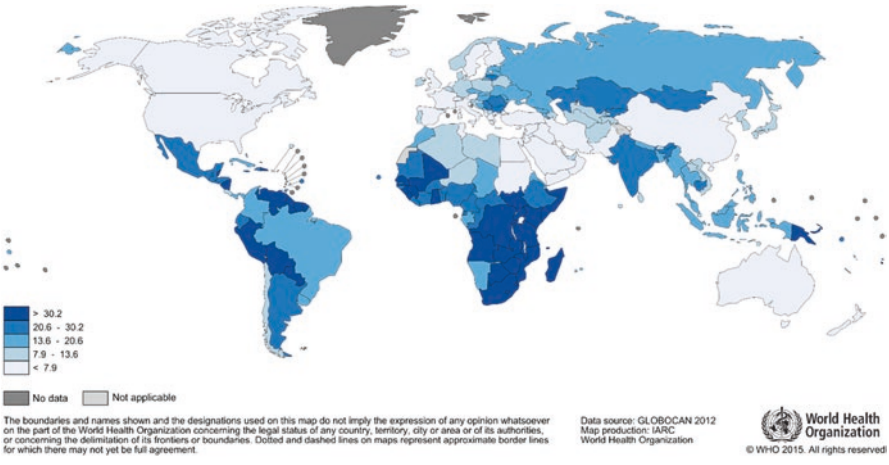

Fig. 6.1 Estimated cervical cancer incidence worldwide in 2012. (From Ferlay [4], with permission)

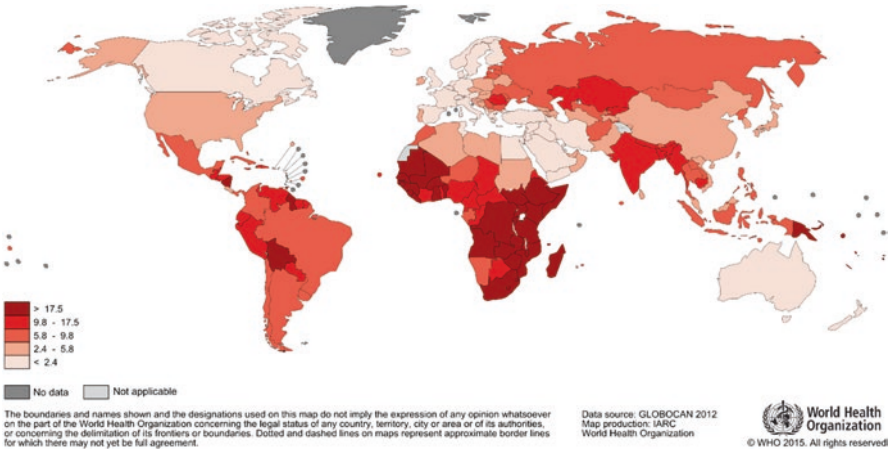

Fig. 6.2 Estimated cervical cancer mortality worldwide in 2012. (From Ferlay [4], with permission)

Epidemiology of HIV Infection

A cluster of cases of *Pneumocystis* pneumonia reported in men in Los Angeles in 1981 heralded what would soon be known as acquired immune deficiency syndrome (AIDS) [5]. Over the next few years, the causative retrovirus was isolated, the nomenclature human immunodeficiency virus (HIV) was agreed upon, and the global nature of the pandemic became apparent [6, 7]. In 1990 a single-center series of 114 patients with preinvasive or invasive cervical neoplasia in the context of HIV infection provided an early description of the poorer prognosis of cervical cancer in this group. The HIV-infected women had more advanced disease, and their cervical cancer persisted or recurred despite therapy in all cases compared to 37% of HIV-negative women [8].

With the advent of combination antiretroviral therapy (cART) in the late 1990s, HIV infection became a chronic, manageable condition [9, 10]. Today, with access to treatment, adults living with HIV can enjoy a life expectancy approximating that of the general population [11], including those living in resource-limited settings [12]. However, despite evidence of benefits to the individual from being on cART even during asymptomatic HIV infection [13], the World Health Organization (WHO) states that of the 36.7 million people estimated to be living with HIV in 2015 (more than half of who were women), only 46% were receiving antiretroviral treatment (http://www.who.int/hiv/data/en/).

WHO data demonstrate that many of the areas where the burden of HIV is highest are also disproportionately affected by cervical cancer. Since the introduction of combination antiretroviral therapy (cART), the standardized incidence rates of almost all AIDS-defining malignancies have fallen substantially. Invasive cervical cancer is an exception, with continued elevated rates of this malignancy seen among those with HIV across multiple studies [14].

Human immunodeficiency virus (HIV) infection is a well-recognized risk factor for the development of cervical cancer. Women living with HIV have been found to be eight times more likely to develop invasive cervical cancers than women who were not HIV infected, and cervical cancer is an AIDS-defining illness [15]. It is not clear how much of the increased risk is attributable to immune suppression and how much this may relate to populations at risk for HIV infection also being at increased risk for acquiring oncogenic strains of HPV [16]. Coinfection with HIV may be associated with a more aggressive course and poorer treatment outcomes than is seen for cervical cancer in HIV-negative women [17]. In addition, HIV infection and the associated immune compromise both pose serious challenges to the clinical management of HIV-positive patients diagnosed with cervical cancer. The overlapping areas where high prevalences of both cervical cancer and HIV infection are seen are represented in Figs. 6.1 and 6.3. These are predominantly in low- and middle-income countries, where access to all of HIV care, HPV vaccine, cervical screening, and cancer treatment is often limited.

There has been a trend toward increased cervical cancer incidence among HIV-infected females in the USA since the introduction of cART (1996–2002; 86.5 cases per 100,000 person-years [PY]) compared to the years 1990–1996 (64.2 cases per 100,000 [PY]) (relative risk [RR] = 1.41, 95% CI = 0.81 to 2.46) [18]. The high incidence of cervical cancer in the cART era may be attributable to the increased longevity in the HIV-infected population.

The median age of occurrence of cervical cancer is reported to be around a decade lower in patients infected with HIV compared to the general population, with the median age being 40 and 52 years, respectively [19]. Some studies report that patients who test HIV positive usually have more advanced stages of cervical

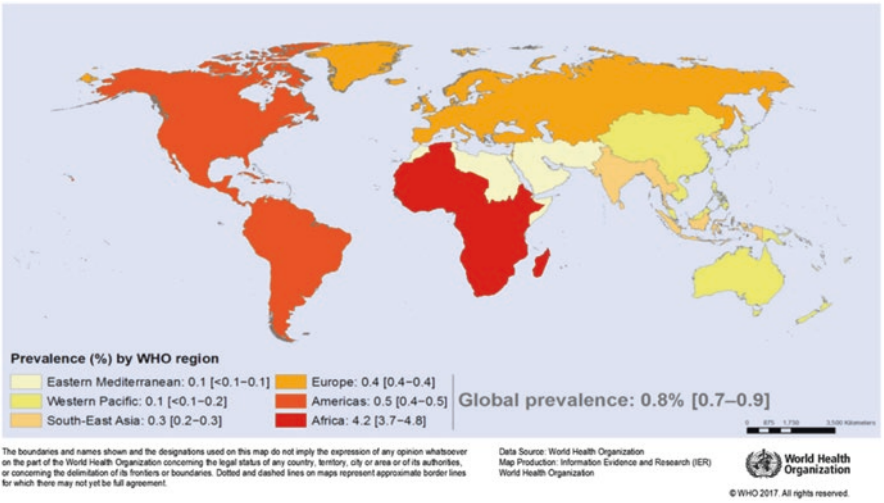

Fig. 6.3 Prevalence of HIV among adults aged 15–49, 2016, by WHO region. (From http://www.who.int/gho/hiv/hiv_013.jpg, with permission)

cancer at presentation than HIV-negative patients [8]. However, a systematic review reported by Atara Ntekim et al. showed no major differences in the proportion of patients presenting with early (stages 1–IIA) vs late (IIB–IVA) stage disease [19].

Etiology

Papillomaviruses are a group of small non-enveloped DNA tumor viruses with a virion size of ~55 nm in diameter [20]. There are approximately 40 different human papilloma virus (HPV) subtypes identified, and these viruses preferentially infect squamous epithelia. The low-risk types (types 6 and 11) are largely responsible for genital warts or condylomatous lesions. Certain high-risk strains of human papilloma virus (HPV), such as HPV-16 and HPV-18, have been recognized to have oncogenic potential and are known to be causative agents of cervical cancer [21]. HPV infections have also been associated with cancers of the anus, vulva, vagina, penis, and head and neck. These high-risk strains cause 70% of cervical cancers and precancerous lesions.

Genital HPV infections are the most common sexually transmitted infections in women and have a peak prevalence between ages 18 and 25. Most HPV infections clear spontaneously without any intervention within a few months after acquisition and about 90% clear within 2 years. A small proportion of infections with certain types of HPV, even in immunocompetent women, can persist (i.e., they are detected for greater than 1 year). It is the persistent high-risk HPV infections that may cause changes in the cervix epithelium leading to the development of cervical cancer precursors – HSIL also known as cervical intraepithelial neoplasia (CIN) 2 or 3 and possible progression to invasive cancer. Factors contributing to persistence and clearance of infection are still unclear. A prospective study of 1728 women by N Munoz et al. found no association between clearance and the women's age or HPV type, with the exception of lower clearance for HPV-16 subtype in women under 30 years of age. However this study did show viral load to be inversely associated with clearance, suggesting that viral load could be a key determinant of persistence [22].

Several studies have documented a higher prevalence of HPV infections in HIV-positive women compared with those who are HIV-uninfected [23]. The prevalence and distribution of prinicipal HPV types involved in cervical cancer carcinogenesis are generally reported to be similar in HIV-infected and non-infected women, although a tendency toward a lower HPV-16 and a higher HPV-18 prevalence in invasive cervical carcinoma has been detected in HIV-positive women, and infection with multiple HPV subtypes is also more common [24]. It is proposed that there are interactions starting at the molecular and cellular level, allowing each infection to promote acquisition and amplification of the other. In ex vivo models of oral and cervical epithelial cells in tissue explants from HIV-uninfected patients, the synergism between HIV proteins (tat and gp120) and cytokines produced as a response to introduction of HIV infection (TNF-a and IFN-gamma) induces disruption of

epithelial tight junctions and potentiated HPV penetration into the basal epithelial cells. HPV displays tropism for these cells and thus the infection is facilitated [25].

The link between HPV, HIV, and cervical cancers is becoming better understood and attributed to enhanced HPV carcinogenesis in the setting of HIV-related immunosuppression, as well as more frequent infections with multiple- and/or high-risk HPV subtypes in HIV-positive women [16]. HIV promotes HPV infection by several mechanisms. Firstly both these infections are transmitted sexually. Secondly, the chronic and progressive immune suppression caused by HIV infection is associated with increased rates of persistent HPV infection, with women with low CD4+ cell counts having the highest prevalence of HPV infection. This in turn results in higher HPV viral load in HIV-infected patients compared to their uninfected counterparts, less clearance of the virus, and more persistent infection. In a similar way, HPV infection also favors increased rates of HIV acquisition by causing disruption to genital mucosal integrity allowing HIV to enter more easily. It has been shown that the E7 protein of HPV-16 potentiates increased permeability of genital mucosa to HIV by downregulating an epithelial adhesion molecule called E-cadherin [26].

Screening and Prevention in HIV-Infected Women

Screening

Strategies for cervical cancer prevention and screening in HIV-infected women are extremely important. The currently used modalities for cervical cancer screening include the cytology-based Papanicolaou test (Pap test), HPV DNA testing, and visual inspection with acetic acid (VIA) or Lugol's iodine (VILI). Regardless of the method employed in screening, it is crucial to expand access to all HIV-infected women and obtain a high follow-up rate from the point of screening through the course of treatment, given their heightened risk of disease and poorer prognosis.

Current recommendations as per NIH guidelines for screening in HIV-infected individuals include the following [27]:

> In adolescent women, whether HIV infection is acquired via sexual or perinatal exposure, the first cervical cancer screening with cytology is recommended to start within the first year of sexual exposure rather than delay to age 21, as is the guideline for normal-risk women. For women with newly diagnosed HIV infection, the first cervical cancer screening is recommended to start at the time of HIV diagnosis with a repeat in 6 or 12 months. The WHO guidelines recommended that all women with HIV should undergo HPV testing [28]. Some guidelines recommend that the length of screening can be extended to every 3 years after three consecutive negative annual screening tests, if testing for HPV is also negative.

Follow-up colposcopy is recommended for evaluation of all abnormal cytological screening results in HIV-infected women, except for atypical squamous cells of undetermined significance (ASC-US), which should be evaluated as in HIV-negative women, where colposcopy is recommended if reflex HPV testing is positive. For women older than 30 years, either cervical cytology testing alone or co-testing with

cytology plus HPV testing is recommend, although co-testing appears preferable. In this age group, either abnormal cytology or positive HPV testing should prompt additional assessment with colposcopy and biopsies as indicated. Cervical cancer screening in HIV-infected women is recommended to continue throughout a woman's lifetime rather than end at a certain age as in the general population.

Several studies have been done to explore the cost-effectiveness of various screening strategies [29]. Cytology-based screening methods and routine colposcopies increase the need for human, financial, and material resources. This is more of a concern in low- and middle-income countries where limited resources for pathological diagnosis contribute to a significant delay between the primary screening visit and the subsequent treatment. This leads to patients being lost to follow-up and in the long term results in disease progression.

The visual inspection with acetic acid (VIA) approach for screening prior to treatment of cervical precancerous lesions in the context of a "screen-and-treat" protocol in low- and middle-income countries is currently recommended in WHO guidelines [30]. The strengths of visual inspection methods include the quick availability of the test results, which allows provision of prompt treatment for screen-positive women. This allows health services to negate the high dropout rates associated with the multiple-visit, cytology-based screening approach.

The main limitation of this technique is the subjectivity of the diagnosis, which is influenced by the examiner's qualitative judgment and the environmental conditions, in which the examination takes place. This subjectivity results in false-positive and false-negative test results, which limit the technique's specificity and sensitivity. The high rates of false-negative diagnoses represent one of the main challenges to the use of this technique for primary screening [31]. However, as same-day treatment with procedures such as cryotherapy or thermocoagulation has proven to be feasible and well accepted by patients, one way to reduce loss to follow-up is to treat all women whose diagnosis is suspicious for a cervical premalignant lesion, although at the cost of increasing the risk of overtreatment.

Treatment of Women with Abnormal Cervical Cytology

HIV-positive women are at increased risk of developing abnormal cervical cytology with prevalence rates of 25% and reported cumulative risks of abnormal cytology of 77% over 10 years [32]. Women with HIV and abnormal cervical cytology should be managed by a clinician who is experienced in both colposcopy and the treatment of cervical cancer precursors. In general, the treatment algorithm is similar to that in non-HIV-infected women, as described in other chapters of this book and should follow standard guidelines such as those of the American Society for Colposcopy and Cervical Pathology (ASCCP) [33]. For women found to have LSIL or worse, referral for colposcopy is recommended. HIV-infected women who are using cART with low viral loads and stable CD4 levels should be managed similarly to non-HIV-infected women [34]. However, it is reported that regression of low-grade lesions is less common in HIV-infected women than non-HIV infected (approximately 30%

cf >70%), and so careful follow-up is required [35]. The use of cART appears to be associated with increased rates of regression and reduced rates of recurrence; hence a diagnosis of CIN is a relative indication to commence cART in women who are not already accessing this [27].

Women with satisfactory colposcopy and biopsy-confirmed HSIL or CIN 2–3 can be treated with either ablation (i.e., cryotherapy or laser) or excision (e.g., loop electrosurgical excision procedure, cold knife conization) of the entire cervical transformation zone, whereas women with unsatisfactory colposcopy or recurrent high-grade CIN should be treated only with excisional methods. Some guidelines, such as those from Australia, recommend that only excisional methods for treatment should be used in women with HIV [36]. Unfortunately, the recurrence rates of HSIL in HIV-positive women following these procedures are reported to be over 50% in some studies and significantly higher than in HIV-negative women [37]. The risk of recurrence also appears to correlate with the degree of immunosuppression, with a recurrence rate of up to 87% reported in a study of severely immunocompromised women (CD4 lymphocyte counts <200 cells/microL), in whom progression from CIN to invasive cancer can occur rapidly [38]. One small randomized trial in 101 HIV-infected women demonstrated that recurrence rates could be reduced by the application of topical 5-FU cream following treatment of CIN 2–3 compared to observation alone [39]. However, this finding has not been replicated in a follow-up study, and hence its use is not recommended consistently in treatment guidelines. Hysterectomy may be recommended for treatment of recurrent or persistent biopsy-confirmed high-grade CIN in women who do not wish to retain fertility. However, for other women ongoing monitoring with cervical cytology and colposcopy at regular intervals is recommended. eg 3 monthly.

Prevention

The use of HPV vaccination for the prevention of cervical cancer and other HPV-related disease is recommended in HIV-infected women, although no studies have reported on the efficacy of HPV vaccination in HIV-infected individuals. Several studies have been completed on the safety and immunogenicity of the bivalent and quadrivalent vaccines in HIV-infected individuals, and given the increased risk of HPV-associated malignancies in this population, vaccination is therefore recommended [40, 41]. Vaccination would ideally occur prior to the onset of sexual activity and is recommended for HIV-infected girls and boys from the age of 9. However, vaccination is still recommended up to the age of 45 in women (including those with HIV infection who have not previously been vaccinated) to provide protection against HPV subtypes they have not yet acquired. Three doses of the quadrivalent vaccine are recommended until further data are available about the safety and efficacy of the 9-valent vaccine in the HIV-infected population.

Treatment of Invasive Cervical Cancer in HIV-Positive Patients

Women who are found to have invasive cervical cancer should be managed by a gyne-oncologist and a multidisciplinary team that includes the specialist who is managing their HIV. Recommended treatment options are based on the FIGO cancer stage and performance status of the patient and are similar to those recommended for women without HIV. The various treatment modalities that are currently employed in the treatment of cervical cancer include surgery, radiotherapy, and chemotherapy either alone or in combination. Unfortunately, delivery of optimal care is often hindered by significant resource constraints in the low- and middle-income countries with the highest burden of disease.

Women with microscopic disease (stage 1A1) without LVSI may be treated with cone biopsy or trachelectomy if fertility preservation is desired, or simple hysterectomy if the patient does not wish to preserve fertility. Women with non-bulky stage 1A2, 1B1, and IIA disease may be treated with radical hysterectomy plus bilateral lymph node dissection. Since radiotherapy (RT) and surgery are equally effective in early stages, surgery should only be considered in patients with earlier stages (up to FIGO IIA) without risk factors necessitating adjuvant therapy, which results in a multimodal therapy without improvement of survival but increased toxicity. Otherwise, women with stage 1B2 disease or higher are generally recommended to receive treatment with chemoradiation involving both external beam radiotherapy with concurrent weekly cisplatin and brachytherapy [42].

Unfortunately there is a lack of high-quality evidence on which to base recommendations about the need for specific treatment modifications in patients with HIV and cervical cancer. In general, patients who are treated with effective cART appear to have higher rates of treatment completion. Hence it is recommended that cART should be commenced at cervical cancer diagnosis in HIV-positive women who are not already accessing this therapy, to ensure less toxicity from cancer therapies and better treatment completion [19].

Radiation Treatment of Cervical Cancer in HIV-Positive Patients

Radiation therapy in locally advanced cervical cancer forms the main backbone of treatment. This, however, has been reported in one study to be associated with a sevenfold increase in multisystemic toxicities in HIV-positive patients compared with HIV-seronegative patients: this included the skin, gastrointestinal (GIT), and genitourinary tract systems (GUT). This study also showed that HIV infection was an independent risk factor for treatment interruptions (adjusted relative risk 2.2). In addition, about 19% of the patients had residual tumor at 4 and 7 months post-EBRT. HIV infection was independently and significantly associated with sixfold higher risk of residual tumor post-EBRT [43].

HIV patients with malignancy have also been reported to have impaired ability of the mucosa to repair radiation damage. With regard to cervical cancer, it is likely that the tissues with mucosal lining close to the treatment fields like the urinary

bladder and gastrointestinal tract (GIT) might be affected in a similar way. This pattern of mucosal reaction is attributable to the low immune status of the patients. Hence access to excellent supportive care for management of radiation toxicities is essential. In addition, if a woman with HIV develops diarrhea during radiotherapy treatment, it is important to try and distinguish this being a side effect of the radiation or one of the other causes of diarrhea seen in HIV infection, including opportunistic infections.

At the University of Miami, where there is a relatively high incidence of both HIV and cervical cancer, both radiotherapy and chemotherapy dose modifications are generally undertaken if the patient's CD4 count is below 200 cells/uL. The daily fraction size is reduced to 1.5Gy, and the weekly cisplatin is reduced to 30–35 mg/m^2. These modifications are observed to result in tolerance similar to that of HIV-negative patients receiving standard dose therapy [44]. Further prospective data is required to validate these observations and guide optimal chemoradiation dosing in immunocompromised hosts.

Challenges in Management in Low- and Middle-Income Countries

While good treatment outcomes for locally advanced disease are possible for many women with HIV, in general treatment outcomes in the setting of HIV are reported to be less good than in women without HIV [17]. The reasons for this are undoubtedly multifactorial and partly relate to the challenges of delivering healthcare in disadvantaged communities that have the highest burden of both HIV and cervical cancer. Women are more likely to present with advanced-stage disease, and social disadvantage may prevent women from attending for treatment even if it is available. One recent study reports a similar rate of initial treatment response in women with HIV, but a higher rate of subsequent relapse, pointing to the role of an intact immune system in control of residual tumor burden among treated cervical cancer patients [45].

Some centers may not be able to offer recommended treatment to all patients due to lack of sufficient access to external beam radiotherapy, brachytherapy, chemotherapy, and cART. One clear major barrier to optimal treatment of cervical cancer, and indeed many cancers, is access to radiation therapy. A recent analysis of radiation therapy infrastructure in 139 low- and middle-income countries found that only 4 (2.87%) have the requisite number of teletherapy units and that 55 (39.5%) have no radiation facilities. Patient access to radiation therapy in the remaining 80 countries ranged from 2.3% to 98.8% (median, 36.7%) [46]. The resource demand in low- and middle-income countries is rising in a steadfast manner, and collaborative public health approaches need to address this concern in a timely manner, in order to narrow this gap and disparity.

The American Society of Clinical Oncology has put forth evidence-based treatment recommendations for cervical cancer based on four resource tiers which have stratifed countries into basic, limited, enhanced, and maximal resource settings [47]. The guideline recommendations were based on a systematic literature review

and developed by consensus opinion of a multidisciplinary panel of experts in cancer control from a range of different countries, including specialists from gynecologic oncology, medical and radiation oncology, health economics, obstetrics, gynecology, and palliative care.

For each setting, and for each stage of cervical cancer, the guideline recommends optimal therapy and palliative care but also puts forward different options where there is a lack of access to the standard therapy. For example, in basic settings where patients cannot be treated with radiation therapy, extra fascial hysterectomy either alone or after neoadjuvant chemotherapy may be an option for women with stage IA1 to IVA cervical cancer. Additionally, in limited resource settings where there is no brachytherapy available, the ASCO Expert Panel recommends extra fascial hysterectomy or its modification for women who have residual tumor 2–3 months after concurrent chemoradiation. Access to lower doses of radiotherapy for palliation of local symptoms, such as pain or bleeding, in addition to opioid analgesia is also recommended.

Chemotherapy Treatment in Women with HIV and Cervical Cancer

In general, it appears that concurrent platinum-based chemotherapy can be safely given to women with HIV as part of chemoradiation treatment for cervical cancer, in close collaboration with the physician managing the HIV. External beam radiotherapy is usually administered with weekly cisplatin 40 mg/m^2 given intravenously for a total of 4–6 weeks. In patients with low CD4 counts, dose reductions may be considered, and in patients with contraindications to cisplatin such as renal impairment due to hydronephrosis, weekly carboplatin AUC 2 can replace cisplatin [48, 49].

A retrospective study done in South Africa showed that the rate of completion of chemotherapy was lower among HIV-positive patients (53.1%) compared with HIV-negative patients (74.6%) [50]. Patients with HIV infection undergoing curative chemoradiation were also found to experience a higher rate of acute hematological toxicity compared to those treated with radiation alone, in terms of both anemia and neutropenia [51]. However renal dysfunction was noted to be the main cause of chemotherapy suspension in HIV-positive patients treated for cervical cancer. The same study also showed that patients who failed to complete chemotherapy had lower median CD4 counts compared to those who completed it, again emphasizing the importance of optimization of HIV management during chemotherapy treatment.

Drug-drug interactions are an important consideration when managing other conditions in people living with HIV infection [52]. Antiretroviral agents (notably non-nucleoside reverse transcriptase inhibitors, protease inhibitors, and the “boosting” agent cobicistat) may cause important drug interactions by inducing and inhibiting various enzymes in the cytochrome P450 family. Several antiretroviral agents are also hepatically metabolized, raising the possibility of interactions with other medications that influence these enzymes. Some medications used to treat

opportunistic infections in HIV patients may also cause clinically important interactions with other drugs. Predicting clinically relevant interactions is made extremely complex, both by the fact that antiretroviral drugs are used in combinations and because most interactions have not been formally studied. There are several online tools available to guide medications that are *likely* to be safe or problematic in patients on antiretroviral therapy. A particularly useful (and freely available) resource is provided by the University of Liverpool at http://www.hiv-druginteractions.org/. This site provides an online "interaction checker" where individual drugs can be entered to check for likely interactions. In addition, up-to-date charts of likely interactions between individual antiretroviral agents and medications used to treat common comorbidities (including a cytotoxic and an analgesic chart) are provided at http://www.hiv-druginteractions.org/treatment_selectors.

Among the cytotoxic agents commonly used in the management of cervical cancer, carboplatin is relatively free from interactions with antiretroviral agents. Cisplatin exposure may be increased if patients are using protease inhibitors as part of their antiretroviral regimen, with potential need for enhanced monitoring and/or dose reduction. In both cases, there is a potential risk of additive nephrotoxicity in patients using tenofovir and hematological toxicity in those using zidovudine. Paclitaxel exposure is likely to be increased among those using protease inhibitors or cobicistat as part of their antiretroviral regimen. Predicted interactions between this drug and non-nucleoside reverse transcriptase inhibitors are more complex, with efavirenz expected to increase and etravirine to decrease paclitaxel exposure, and paclitaxel itself may cause reduced levels of etravirine or rilpivirine. Paclitaxel is also expected to result in reduced levels of maraviroc and integrase inhibitors (raltegravir and dolutegravir). Information about the safety of using bevacizumab to treat cervical cancer in the setting of HIV has not been published; however some data is available to suggest that bevacizumab, which is metabolized and eliminated via the reticuloendothelial system, can be safely used in the treatment of other HIV-associated malignancies such as Kaposi's sarcoma [53].

If significant renal impairment develops during chemotherapy, doses of some antiretroviral agents may need to be reduced (notably nucleoside and nucleotide reverse transcriptase inhibitors). Where treatment is likely to cause prolonged or severe loss of appetite, nausea, or vomiting, the use of antiretroviral agents that need to be taken with food for absorption should be avoided where possible. Detailed charts of dose adjustments in the setting of renal impairment as well as food restrictions with antiretrovirals are available on the Liverpool site.

Overlapping toxicities between antiretroviral agents and oncology drugs may also need to be considered. For example, the manufacturer recommends against co-administering rilpivirine (a non-nucleoside reverse transcriptase inhibitor) with agents that may cause prolongation of the QT interval. Peripheral neuropathy is a common consequence of both HIV infection and also some HIV treatments – notably stavudine and didanosine. Although these agents are now rarely used in well-resourced settings, the available data suggests an ongoing high prevalence of neuropathy exists among those living with HIV [54]. A heightened awareness of neuropathy risk in any patient with HIV who is offered cytotoxic chemotherapy is

recommended, including warning patients about this possibility, given the disabling and typically permanent nature of this complication.

The complexity of optimally using cytotoxic therapy with combination antiretrovirals make it highly desirable that treatment decisions should be made with input from all of the oncologist, the treating HIV clinician, and an experienced pharmacist. Careful consideration of potentially problematic interactions before treatment commences, and adjustment of antiretroviral regimens if necessary/practical, is an essential part of optimizing both the efficacy and tolerability of therapy.

Awareness and Avoidance of Stigma in the Care of Women with HIV and Cancer

Stigma remains a very real issue for people living with HIV around the world, including in healthcare settings. Numerous adverse events related to stigma have been documented, including unwanted disclosure of the patient's HIV status to others, inappropriate or excessive use of precautions by staff fearing infection, and even delaying or refusal of care [55–58].

Some authors have found HIV-associated stigma to be associated with lack of education and/or experience of managing individuals with HIV infection, as well as healthcare workers' personal beliefs**.** Potentially reversible causes of stigma include a lack of awareness among staff of what stigma is and why it is harmful, unrealistic staff fears of HIV infection, and prejudice surrounding beliefs associating HIV with particular behaviors. Importantly, simple educational interventions may reduce the stigmatizing behavior among healthcare staff [59, 60].

A cancer diagnosis typically brings with it the need for women to disclose their HIV status to a new group of healthcare professionals and in some cases attendance at a new healthcare facility. It is important to recognize both the anxiety this is likely to cause in the HIV-positive woman diagnosed with cervical cancer and also the fact that patient concerns may be well founded. Clear documentation of who is aware of the woman's HIV status and particular care surrounding confidentiality is important. This may include (but is not limited to) avoiding mentioning HIV in discussions with family members or other visitors unless the patient explicitly permits this, careful attention to storage of medical records (including ensuring neither paper nor electronic records are visible to others in wards or clinics), using the same infection prevention processes (i.e., truly universal precautions) for women with HIV as for others, and ensuring a woman's HIV status is not disclosed to those without a clinical basis for needing this knowledge. In settings with very limited experience of caring for people with known HIV infection, education may allay fears and improve staff awareness of HIV stigma and the importance of reducing this. Clear collaboration with the clinicians involved in managing the woman's HIV, who often have a long-term and trusting relationship with the patient, may have benefits in enhancing the therapeutic relationship with the oncology team, as well being critical in ensuring the best possible care.

Conclusions/Summary

Cervical cancer incidence is increased among women living with HIV, and the prognosis is typically reported to be poorer than among their HIV-uninfected counterparts, making this a priority population for prevention (HPV vaccination) and early diagnosis (cervical screening) strategies wherever possible. When established cervical cancer is diagnosed in a woman with HIV infection, management is broadly similar to that recommended for HIV-uninfected women with similar disease. However, women with HIV, particularly those with more advanced immune suppression, may be at increased risk for recurrent disease as well as being less able to tolerate recommended doses of chemotherapy and radiotherapy. Unfortunately, the greatest burden of both HIV infection and cervical cancer occurs in low- and middle-income countries, where access to healthcare services (including all of HIV care, definitive cancer therapy, and palliative care) may be limited. Further, issues surrounding HIV stigma in the healthcare setting are real and may substantially impact on women's ability and willingness to access appropriate care, even where this is available.

The available evidence suggests that optimal HIV management with cART improves prognosis for HIV-positive women with cervical cancer, and wherever possible, this should be part of care. Careful attention to avoid problematic drug interactions and cumulative toxicities between cancer treatments and antiretroviral agents is essential and is facilitated by collaboration between the oncology team, the treating HIV clinician, and an experienced pharmacist. Further research is needed to establish the optimal management of cervical cancer in HIV-positive women who have had access to optimal HIV care (cART from soon after diagnosis, even if asymptomatic) and who live in areas where they have affordable access to all appropriate modalities of cancer treatment.

References

1. Arbyn M, Castellsague X, de Sanjose S, et al. Worldwide burden of cervical cancer in 2008. Ann Oncol Off J Eur Soc Med Oncol. 2011;22(12):2675–86.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69–90.
3. Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol Off J Am Soc Clin Oncol. 2006;24(14):2137–50.
4. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer incidence and morality worldwide. ARC CancerBase No. 11 [internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr. Accessed 28 Aug 2017.
5. (CDC) CfDC. Pneumocystic pneumoniae – Los Angeles. MMWR Morb Mortal Wkly Rep. 1981;30(21):250–2.
6. Marx JL. Strong new candidate for AIDS agent. Science. 1984;224(4648):475–7.
7. Case K. Nomenclature: human immunodeficiency virus. Ann Intern Med. 1986;105(1):133.
8. Maiman M, Fruchter RG, Serur E, Remy JC, Feuer G, Boyce J. Human immunodeficiency virus infection and cervical neoplasia. Gynecol Oncol. 1990;38(3):377–82.

9. Collier AC, Coombs RW, Schoenfeld DA, AIDS Clinical Trials Group, et al. Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. N Engl I Med. 1996;334(16):1011–7.
10. D'Aquila RT, Hughes MD, Johnson VA, National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group Protocol 241 Investigators, et al. Nevirapine, zidovudine, and didanosine compared with zidovudine and didanosine in patients with HIV-1 infection. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 1996;124(12):1019–30.
11. van Sighem AI, Gras LA, Reiss P, Brinkman K, de Wolf F, Anoc s. Life expectancy of recently diagnosed asymptomatic HIV-infected patients approaches that of uninfected individuals. AIDS. 2010;24(10):1527–35.
12. Mills EJ, Bakanda C, Birungi J, et al. Life expectancy of persons receiving combination antiretroviral therapy in low-income countries: a cohort analysis from Uganda. Ann Intern Med. 2011;155(4):209–16.
13. Group ISS, Lundgren JD, Babiker AG, et al. Initiation of antiretroviral therapy in early asymptomatic HIV infection. N Engl J Med. 2015;373(9):795–807.
14. Rubinstein PG, Aboulafia DM, Zloza A. Malignancies in HIV/AIDS: from epidemiology to therapeutic challenges. AIDS. 2014;28(4):453–65.
15. Clifford GM, Polesel J, Rickenbach M, et al. Cancer risk in the Swiss HIV Cohort Study: associations with immunodeficiency, smoking, and highly active antiretroviral therapy. J Natl Cancer Inst. 2005;97(6):425–32.
16. Clifford GM, de Vuyst H, Tenet V, Plummer M, Tully S, Franceschi S. Effect of HIV infection on human papillomavirus types causing invasive cervical cancer in Africa. J Acquir Immune Defic Syndr. 2016;73(3):332–9.
17. Dryden-Peterson S, Bvochora-Nsingo M, Suneja G, et al. HIV infection and survival among women with cervical cancer. J Clin Oncol Off J Am Soc Clin Oncol. 2016;34(31):3749–57.
18. Biggar RJ, Chaturvedi AK, Goedert JJ, Engels EA, Study HACM. AIDS-related cancer and severity of immunosuppression in persons with AIDS. J Natl Cancer Inst. 2007;99(12):962–72.
19. Ntekim A, Campbell O, Rothenbacher D. Optimal management of cervical cancer in HIV-positive patients: a systematic review. Cancer Med. 2015;4(9):1381–93.
20. Zheng ZM, Baker CC. Papillomavirus genome structure, expression, and post-transcriptional regulation. Front Biosci. 2006;11:2286–302.
21. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003;348(6):518–27.
22. Munoz N, Hernandez-Suarez G, Mendez F, et al. Persistence of HPV infection and risk of high-grade cervical intraepithelial neoplasia in a cohort of Colombian women. Br J Cancer. 2009;100(7):1184–90.
23. Ng'andwe C, Lowe JJ, Richards PJ, Hause L, Wood C, Angeletti PC. The distribution of sexually-transmitted human papillomaviruses in HIV positive and negative patients in Zambia, Africa. BMC Infect Dis. 2007;7:77.
24. Darwich L, Canadas MP, Sirera G, et al. Human papillomavirus genotype distribution and human papillomavirus 16 and human papillomavirus 18 genomic integration in invasive and in situ cervical carcinoma in human immunodeficiency virus-infected women. Int J Gynecol Cancer Off J Int Gynecol Cancer Soc. 2011;21(8):1486–90.
25. Tugizov SM, Herrera R, Chin-Hong P, et al. HIV-associated disruption of mucosal epithelium facilitates paracellular penetration by human papillomavirus. Virology. 2013;446(1–2):378–88.
26. Laurson J, Khan S, Chung R, Cross K, Raj K. Epigenetic repression of E-cadherin by human papillomavirus 16 E7 protein. Carcinogenesis. 2010;31(5):918–26.
27. Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents. Accessed 02/09/2017.
28. Organisation WH. WHO guidelines for screening and treatment of precancerous lesions for cervical cancer prevention2013. http://apps.who.int/iris/bitstream/10665/94830/1/9789241548694_eng.pdf. Accessed.

29. Lince-Deroche N, Phiri J, Michelow P, Smith JS, Firnhaber C. Costs and cost effectiveness of three approaches for cervical cancer screening among HIV-positive women in Johannesburg, South Africa. PLoS One. 2015;10(11):e0141969.
30. WHO. Comprehensive cervical cancer control: a guide to essential practice. 2nd ed. Geneva: WHO Press; 2014.
31. Qiao L, Li B, Long M, Wang X, Wang A, Zhang G. Accuracy of visual inspection with acetic acid and with Lugol's iodine for cervical cancer screening: meta-analysis. J Obstet Gynaecol Res. 2015;41(9):1313–25.
32. Massad LS, Seaberg EC, Wright RL, et al. Squamous cervical lesions in women with human immunodeficiency virus: long-term follow-up. Obstet Gynecol. 2008;111(6):1388–93.
33. Wright TC Jr, Massad LS, Dunton CJ, et al. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. Am J Obstet Gynecol. 2007;197(4):346–55.
34. Ahdieh-Grant L, Li R, Levine AM, et al. Highly active antiretroviral therapy and cervical squamous intraepithelial lesions in human immunodeficiency virus-positive women. J Natl Cancer Inst. 2004;96(14):1070–6.
35. Delmas MC, Larsen C, van Benthem B, European Study Group on Natural History of HIV Infection in Women, et al. Cervical squamous intraepithelial lesions in HIV-infected women: prevalence, incidence and regression. AIDS. 2000;14(12):1775–84.
36. Brand A, Hammond I, Pather S, Roeske L, Wrede CD. Cancer council Australia cervical Cancer screening guidelines working party. 16. Screening in immune-deficient women. http://wiki.cancer.org.au/australia/Clinical_question:Screening_in_immune-deficient_women. Accessed 1/9/2017.
37. Reimers LL, Sotardi S, Daniel D, et al. Outcomes after an excisional procedure for cervical intraepithelial neoplasia in HIV-infected women. Gynecol Oncol. 2010;119(1):92–7.
38. Heard I, Potard V, Foulot H, Chapron C, Costagliola D, Kazatchkine MD. High rate of recurrence of cervical intraepithelial neoplasia after surgery in HIV-positive women. J Acquir Immune Defic Syndr. 2005;39(4):412–8.
39. Maiman M, Watts DH, Andersen J, Clax P, Merino M, Kendall MA. Vaginal 5-fluorouracil for high-grade cervical dysplasia in human immunodeficiency virus infection: a randomized trial. Obstet Gynecol. 1999;94(6):954–61.
40. Levin MJ, Moscicki AB, Song LY, et al. Safety and immunogenicity of a quadrivalent human papillomavirus (types 6, 11, 16, and 18) vaccine in HIV-infected children 7 to 12 years old. J Acquir Immune Defic Syndr. 2010;55(2):197–204.
41. Wilkin T, Lee JY, Lensing SY, et al. Safety and immunogenicity of the quadrivalent human papillomavirus vaccine in HIV-1-infected men. J Infect Dis. 2010;202(8):1246–53.
42. Marth C, Landoni F, Mahner S, et al. Cervical cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol Off J Eur Soc Med Oncol. 2017;28(suppl_4):iv72–83.
43. Gichangi P, Bwayo J, Estambale B, et al. HIV impact on acute morbidity and pelvic tumor control following radiotherapy for cervical cancer. Gynecol Oncol. 2006;100(2):405–11.
44. Housri N, Yarchoan R, Kaushal A. Radiotherapy for patients with the human immunodeficiency virus: are special precautions necessary? Cancer. 2010;116(2):273–83.
45. Ferreira MP, Coghill AE, Chaves CB, et al. Outcomes of cervical cancer among HIV-infected and HIV-uninfected women treated at the Brazilian National Institute of Cancer. AIDS. 2017;31(4):523–31.
46. Datta NR, Samiei M, Bodis S. Radiation therapy infrastructure and human resources in low- and middle-income countries: present status and projections for 2020. Int J Radiat Oncol Biol Phys. 2014;89(3):448–57.
47. Chuang LT, Temin S, Camacho R, et al. Management and care of women with invasive cervical cancer: American Society of Clinical Oncology resource-stratified clinical practice guideline. J Glob Oncol. 2016;2(5):311–40.

48. Sebastiao AM, da Silva Rocha LS, Gimenez RD, et al. Carboplatin-based chemoradiotherapy in advanced cervical cancer: an alternative to cisplatin-based regimen? Eur J Obstet Gynecol Reprod Biol. 2016;201:161–5.
49. Au-Yeung G, Mileshkin L, Bernshaw DM, Kondalsamy-Chennakesavan S, Rischin D, Narayan K. Radiation with cisplatin or carboplatin for locally advanced cervix cancer: the experience of a tertiary cancer centre. J Med Imaging Radiat Oncol. 2013;57(1):97–104.
50. Simonds HM, Wright JD, du Toit N, Neugut AI, Jacobson JS. Completion of and early response to chemoradiation among human immunodeficiency virus (HIV)-positive and HIV-negative patients with locally advanced cervical carcinoma in South Africa. Cancer. 2012;118(11):2971–9.
51. Simonds HM, Neugut AI, Jacobson JS. HIV status and acute hematologic toxicity among patients with cervix cancer undergoing radical chemoradiation. Int J Gynecol Cancer Off J Int Gynecol Cancer Soc. 2015;25(5):884–90.
52. Rudek MA, Flexner C, Ambinder RF. Use of antineoplastic agents in patients with cancer who have HIV/AIDS. Lancet Oncol. 2011;12(9):905–12.
53. Uldrick TS, Wyvill KM, Kumar P, et al. Phase II study of bevacizumab in patients with HIV-associated Kaposi's sarcoma receiving antiretroviral therapy. J Clin Oncology Off J Am Soc Clin Oncol. 2012;30(13):1476–83.
54. Cherry CL, Wadley AL, Kamerman PR. Painful HIV-associated sensory neuropathy. Pain Manag. 2012;2(6):543–52.
55. Mahendra VS, Gilborn L, Bharat S, et al. Understanding and measuring AIDS-related stigma in health care settings: a developing country perspective. SAHARA J J Soc Asp HIV/AIDS Res Alliance. 2007;4(2):616–25.
56. Rutledge SE, Abell N, Padmore J, McCann TJ. AIDS stigma in health services in the Eastern Caribbean. Sociol Health Illn. 2009;31(1):17–34.
57. Cannon Poindexter C. HIV stigma and discrimination in medical settings: stories from African women in New Zealand. Soc Work Health Care. 2013;52(8):704–27.
58. Stutterheim SE, Sicking L, Brands R, et al. Patient and provider perspectives on HIV and HIV-related stigma in Dutch health care settings. AIDS Patient Care STDs. 2014;28(12):652–65.
59. Nyblade L, Stangl A, Weiss E, Ashburn K. Combating HIV stigma in health care settings: what works? J Int AIDS Soc. 2009;12:15.
60. Li L, Wu Z, Liang LJ, et al. Reducing HIV-related stigma in health care settings: a randomized controlled trial in China. Am J Public Health. 2013;103(2):286–92.

Immunotherapy for Precancerous Lesions of the Uterine Cervix

7

Samir A. Farghaly

Uterine cervical cancer is the fourth most common neoplasia in women and the seventh overall. In 2012, there were 528,000 new cases and 266,000 deaths from cervical cancer worldwide, accounting for 7.5% of all female cancer deaths. It is the most frequent gynecological cancer in developing countries [1, 2]. The frequency of cervical cancer after treatment for dysplasia is less than 1% and mortality is less than 0.5% [3]. The increasing incidence of the disease in developing countries is related to the high number of multiple partners, early age at first intercourse, infrequent use of condoms, multiple pregnancies with chlamydia association, and immunosuppression with HIV [4]. It was noted that HIV-infected women have a higher risk and persistence of multiple HPV infections which are associated with increased risk of progression to precancerous cervical lesions compared to HIV-noninfected women [5]. About 10–15% of women have oncogenic HPV types (HPV high risk, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, and 82, and HPV low risk, 6, 11, 40, 42, 43, 44, 54, 61, 72, and 81) [6]. In the United States of America (USA), HPV-16 and HPV-18 types are detected in 70% of high-grade squamous intraepithelial lesions (HGSIL) and in invasive cervical cancer in women [7]. It has been shown that oral contraceptives are associated with increased risk of the disease (administration for >5-year-double risk, >10-year-quadruple risk). In addition, other risk factors such as sexual activity, frequency of gynecological examinations, and medication-free interval time are observed [7, 8]. Interestingly, smoking is thought to have unclear relation to the disease [9]. There are several mechanisms by which cancers can avoid immune defenses. Cancers can directly inhibit immune reactivity by secreting soluble immune inhibitory mediators such as PGE2, TGF-β, and IL-10

S. A. Farghaly (✉)
The Joan and Sanford I. Weill Medical College/Graduate School of Medical Sciences, The New York Presbyterian Hospital-Weill Cornell Medical Center, and Sandra and Edward Meyer Cancer Center, Cornell University, New York, NY, USA

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_7

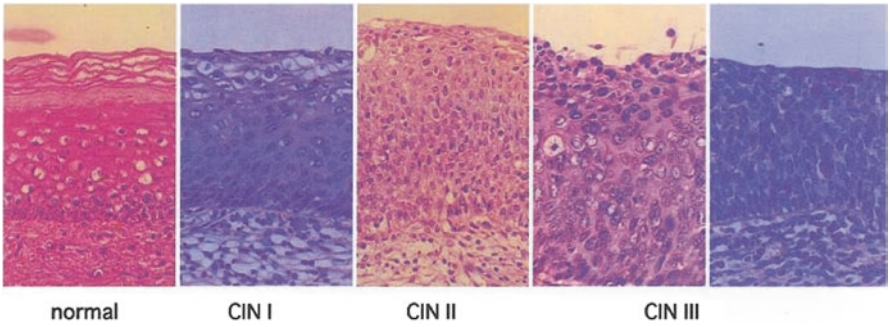

Fig. 7.1 Histopathologic features of cervical intraepithelial neoplasia (CIN) stages (From Fukumoto and Irahara [236], with permission)

[10–12]. They also express checkpoint inhibitory ligands such as PD-L1 that block immune reaction [13]. In addition, inhibition by cancers is mediated by their induction of host immune inhibitory cell populations. These include macrophages, Treg cells, Th2-skewed T-cells, myeloid-derived suppressor cells (MDSC), and $CD34^+$ progenitor cells [14–18]. Within the tumor environment, there are also immune inhibitory endothelial cells and fibroblasts [19, 20]. Histopathologic features of cervical intraepithelial neoplasia (CIN) stages are shown in Fig. 7.1 [21].

Immunological Aspects of Precancerous Lesions of the Uterine Cervix

Noted efforts have been exerted on cancer prevention such as improved diet, smoking cessation, and reduced sun exposure. Less emphasis has been placed on immunological approaches to prevent cancer development or progression prior to when cancers subvert immune defenses. However, an advancement toward this effort is the availability of HPV vaccines, which aim to prevent cervical cancer and can become effective in preventing other HPV-associated malignancies such as squamous cell carcinomas (SCC) of the head and neck [22, 23]. Non-HPV-associated malignancies might be preventable in individuals that are at high risk for development of cancer. In general, premalignant lesions are tissues that can progress to become malignant. Examples of these precancerous tissues include polyps in the colon, actinic keratosis of the skin, dysplasia of the cervix, metaplasia of the lung, and leukoplakias of the mouth. Premalignant lesions of the oral cavity, including leukoplakias and erythroplakias, are routinely screened during dental examinations [24]. Colonoscopies are performed routinely to detect colon polyps which, in turn, reduce colon cancer [25, 26]. Dysplasia of the cervix is routinely screened for by Pap smears [27]. The standard treatment for these premalignant tissues often includes their excision; however such treatment does not remove premalignant cells that have not yet been detected and does not prevent development of secondary lesions. A study compared the immunological microenvironment of intraepidermal

carcinomas, and SCC showed an increased level of T-cells, and mainly $CD8^+$ T-cells, within the lesions compared to the levels of these cells in cancer tissue [28]. In another study, the investigators showed that premalignant oral leukoplakias are infiltrated by $CD3^+$ T-cells, with those containing lower numbers of $CD3^+$ cells having a higher incidence of progression to cancer [29]. It has also been shown that leukoplakias with dysplasia and oral SCC have a higher dendritic Langerhans cell and T-cell content than leukoplakias without dysplasia [30]. The conclusions of these studies suggest that the higher level of immune cell infiltration is indicative of ongoing immune reactivity against premalignant lesions and against cancers. Other studies showed that premalignant oral lesion tissues of patients and of a mouse model of premalignant oral lesions that progress to cancer contained increased levels of Th1 and inflammatory cytokines compared to levels within oral cancers [31]. Studies of the immune phenotypes have shown Barrett's esophageal tissues contain an elevated pro-tumorigenic Th2 immune phenotype, but this shifts to a less activated T-cell phenotype once the cancer is developed that consists of a mixed Th1 and Th2 cytokine profile [32]. Also, infiltration by M2 macrophages and Treg cells was hypothesized to contribute to esophageal cancer development in a rat model of chronic duodenal content reflux esophagitis [33]. Similarly, studies with *Helicobacter pylori*-infected patients having precancerous gastric lesions and *H. pylori*-infected mice concluded that increased myeloid cell infiltration and increased IFN-γ expression may be contributing to progression of lesions toward cancer [34]. Additionally, genetic expression profiles of colon polyp tissues and unaffected colon mucosa of patients having colon polyps showed an overlap of changes in gene expression compared to gene expression profiles of healthy individuals [35]. It was noted that patients with ulcerative colitis had a similar frequency of developing polyps as did healthy controls, although the histological types of polyps differed with an increase in inflammatory (pseudo)polyps [36]. Studies indicating immune involvement in progression of premalignant states toward cancer using the TRAMP mouse model showed the development of hyperplasia, prostatic intraepithelial neoplasia, and carcinoma. The presence of T-cells was shown to facilitate the process of progression [37]. Another study with a murine model of prostatic hyperplasia suggested immune involvement in stimulating prostatic epithelial proliferation, and the inflammatory reaction was mediated by macrophage-derived IL-1 [38]. It was suggested that macrophage recruitment promotes the formation and progression of pancreatic premalignant lesions [39]. Inflammation along the gastrointestinal tract appears to have a closer connection to progression of premalignant states to cancer than what has been described for other sites. Such inflammation-associated disorders with increased risk of cancer include Barrett's esophagus, Crohn's disease, and ulcerative colitis [40, 41]. Levels of inflammatory indicators such a C-reactive protein and IL-6 were shown to be increased in the peripheral blood of subjects with Barrett's esophagus, and these increases were associated with a higher risk of premalignant progressing to esophageal adenocarcinoma [42]. Subjects with premalignant oral lesions have increased levels of inflammatory mediators, TNF-α and IL-6 in their saliva, although salivary levels of these cytokines were shown to be higher in subjects with oral squamous cell carcinoma [43]. It was noted increased

levels of TNF-α in saliva of subjects with premalignant oral lesions and cancer were increased in the serum of these subjects [44]. Other studies showed increased splenic and regional lymph node pro-inflammatory activity with a Th1 and Th17 phenotype in a carcinogen-induced premalignant oral lesion animal model and in the blood of subjects with premalignant oral lesions [40, 45]. Studies to assess the mechanism by which premalignant oral lesion cells alter cytokine levels demonstrated that the stimulation of Th1 and Th17 cell-associated cytokines was through soluble mediators produced by premalignant lesion cells [41, 46]. The induction of some of the inflammatory mediators was blocked by inhibiting cyclooxygenase in premalignant lesion cells, hypothesizing that lesion cell-derived PGE2 could be contributing to some of the systemic inflammation [47]. The immune system is divided into two components: the innate immune system and the adaptive immune system. The latter is further subdivided into humoral immunity and cell-mediated immunity [44]. Innate and adaptive immune systems are intertwined, through several immune cells and cytokines that are involved in both the innate and adaptive immune responses. Innate immune response provides initial defense against pathogens by epithelial barriers, local inflammation and cytokines, complement system and phagocytic cells (neutrophils, monocytes, and macrophages), dendritic cells (DC), and natural killer (NK) cells [48]. NK-cells recognize tumor cells expressing histocompatibility complex (MHC) surface molecules and are responsible for killing these cancer cells by releasing perforin and granzyme that enter the cytoplasm and induce apoptosis [49]. Two functional types of receptors are expressed on the NK-cell surface: stimulatory receptors and inhibitory receptors. Natural killer group 2D (NKG2D) molecule is a known stimulatory receptor [50]. Binding of stress-related ligands on tumor cells with NKG2D stimulates NK-cells and results in secretion of interferon (IFN) gamma and perforin, release of inflammatory cytokines, and induction of apoptosis in cancer cells. Macrophages can phenotypically and functionally be categorized into M1-like, pro-inflammatory, tumor-suppressive macrophages (M1) and M2-like anti-inflammatory tumor-promoting (M2) macrophages [46]. M1 macrophages develop in response to bacterial products, acute inflammation, and IFN-a and recognize tumor cells expressing eat-me molecules at the cell surface. These signals include lipid phosphatidylserine (PS), oxidized PS, oxidized low-density lipoprotein, and calreticulin [51] which are translocated to the tumor cell surface during apoptosis [52]. Interaction between apoptotic tumor cells and these macrophages leads to immune tolerance in a tumor environment. M1 macrophages are also capable of extracellular killing of cancer cells by the release of cytokines, chemokines, and inflammatory mediators. In addition, M2 macrophage produces immunosuppressive cytokines and chemokines that result in alteration of the phenotype and function of local DCs and polarize T-cells to a x2 phenotype which decrease an antitumor immune response [53, 54]. Myeloid-derived suppressor cells (MDSC) hinder an antitumor immune response [55, 56] and are present in tumor microenvironment. Consequently, tumors attract myeloid cells and interfere with their differentiation. Dendritic cells (DCs) are highly specialized in antigen presentation to T-cells and act as bridges between the innate and the adaptive immune system. In cancer, tumor-infiltrating B-cells (TIL-Bs) play a key role

in the B-cell response. There is increasing evidence that the presence of TIL-Bs is associated with favorable clinical outcomes in cancer. In addition, B-cells can potentiate the antitumor response by producing chemokines and cytokines, as they serve as local APCs and organize lymphoid structures in the tumor that sustains the immune response [57]. Whereas B-cells recognize whole molecules and intact pathogens, T-cells possess T-cell receptors (TCR) that recognize small peptide antigens presented by MHC class I or II on the cell surface. Naïve T-cells need to recognize the antigen and receive a co-stimulatory signal to become activated, differentiated, and proliferated into effector cells. Co-stimulatory molecules provide signals which are involved in activating and regulating the development antigen-specific T-cells [58]. There are two major T-lymphocyte populations, CD8+ and CD4+ T-cells, which recognize distinct fragments of antigens and display distinct effector functions. CD8+ cytotoxic T-cells (CTLs) recognize small peptide antigens that are presented in MHC class I molecules on the cells. Ayer's recognition of the abnormally expressed antigen and CD8+ T-cells differentiate into cells that acquire cytolytic capacity, ending with a highly specific mature CTL that can kill the affected cell. CD4+ T-cells recognize antigens presented in MHC class II molecules. In addition to MHC class II expression by immune cells, such as APCs, MHC class II expression occurs in activated CD4+ T-cells and CD8+ T-cells and can be upregulated in epithelial cells in tumor cells [59]. CD4+ T-cell activation is essential for an optimal CD8+ T-cell-mediated immune response [59], either through the classical helper role of CD4+ T-cells that provide cytokine support (IL-2 and IFN-a release) for CD8+ T-cells or by the activation of CD40 expression on APCs which stimulate CD8+ T-cells [60, 61]. CD4+ T-cells can be polarized into multiple different effector T-cell subsets, based on their function and cytokine profile, including type 1 x (x1) helper cells, type 2 x (x2) helper cells, and x17 cells which play an important role in the induction of autoimmunity, but recent evidence suggests that this effector T-cell subset is also involved in tumor immunology by preparing the tumor environment and facilitating tumor-infiltrating CD8+ T-cells and NK-cells [62]. A specialized subtype of CD4+ T-cells distinguished from other subpopulations by their role in immune tolerance is the regulatory T-cell (Treg) subset. Naturally occurring Tregs are directly derived from the thymus, and these highly express CD25 and transcription factor FoxP3. Adaptive Tregs are induced at the periphery and may or may not express FoxP3. Tregs suppress CD8+ CTLs and x1-mediated responses via various known and unknown mechanisms, including the secretion of immunosuppressive cytokines as IL-10 and TGF-β or the consumption of IL-2, thereby inhibiting other T-cells or APCs.

Cancer Immunology

The immune system plays an important role in the development, maintenance, and expansion of cancer. Several numbers of immune cells with different subsets, receptors, cytokines, antibodies, and chemokines contribute to the elimination or promotion of tumor progression. It has been hypothesized that the immune system is able

to recognize, inactivate, and eliminate potentially malignant cells before they establish themselves and form a tumor mass [63–65]. In general, malignant cells are ascribed as the result of genetic changes that occur during cell divisions. Genetic changes may result in the expression of tumor antigens, which make malignant cells immunologically distinguishable from normal cells [66]. There are three interaction processes between tumor cells and immune cells. These three processes include the elimination, equilibrium, and escape phase, representing the fact that the immune system protects the host against tumor development and modulates the immunogenic phenotype of malignant cells (equilibrium phase) and thereby facilitating complete tumor escape from immune attack (escape phase) and uncontrolled tumor growth [67, 68]. Several studies have shown that the nature of tumor-infiltrating T-cells at diagnosis is strongly associated with patient survival in many human cancers [69–73]. The prognostic value of adaptive immune cell infiltration and tumor microenvironment was noted in colorectal cancer, and expressed as an integrated immunoscore, which was based on the type, density, and location of immune cells [74–76]. The role of HPV infections in the development of cervical premalignancies has been recognized [77]. Genital infections with high-risk HPV, particularly HPV type 16 (HPV16), are highly prevalent in young individuals with a lifetime incidence of 80% [78]. The majority of immune competent individuals infected with the virus are able to control and eventually eliminate the viral infection. In most women, an HPV infection is asymptomatic, transient, and cleared within 2 years. Persistent infections with HPV occur in less than 10% of the infected women which increase the risk of development of premalignant cervical lesions [79]. HPV is a non-lytic, circular double-stranded DNA which encodes for six early nonstructural or regulatory genes (E1, E2, and E4–E7) and two late structural proteins (L1 and L2) [80]. These proteins exert specific functions during the different stages of HPV replication which contribute to the development and progression of HPV-associated lesions. Replication of HPV occurs in the supra-basal layer, where E1, E2, and E5 genes are expressed. Oncoproteins E6 and E7 are consistently expressed in the basal cells of the epithelium layer and play an important role in the viral life cycle by modifying the cellular environment and allow viral genome amplification, by driving S-phase reentry in the upper epithelial layers [81, 82]. In case of persistent infection with high-risk HPV, integration of the HPV DNA into the host cell genome might occur and is accompanied with overexpression of E6 and E7 oncoproteins. Persistent high level of expression of E6 and E7 accumulates genetic errors in the host genome, resulting in dysplastic cells which can progress to high-grade intraepithelial lesions or microinvasive carcinoma [83]. Notably, immunosuppressed individuals are known to be at high risk for persistent HPV infections, HPV-associated malignancies, and progression of disease [84, 85]. Undifferentiated keratinocytes at the stratum basale of the epithelium are the primary target for HPV. Keratinocytes express pathogen recognition receptors (PRRs), including the Toll-like receptors (TLRs), nucleotide oligomerization domain-like receptors (NLRs), and retinoic acid-inducible gene 1 (RIG-I)-like receptors (RLRs), which recognize pathogen-associated molecular patterns (PAMPs) on microbes and viruses [86]. TLRs1–3, TLR5, TLR6, TLR10, RIG-I, protein kinase R (PKR), and

MDA5 are expressed irrespective of the differentiation state of keratinocytes, while the expression of TLR9, the PPR that can recognize viral DNA of HPV, only induced layer terminal differentiation [87]. Moreover, HPV infection downregulates a network of genes encoding for the production and secretion of antivirals such as type I interferon and chemotactic and pro-inflammatory cytokines, including IL-1β, which play a major role in activation of adaptive immunity [87, 88]. HPV also attenuates the effector cytokine reaction of infected cells to the exposure to IFN-a and/or TNF-α, allowing transient escape from immune response [89]. Further, HPVs are able to manipulate Langerhans cells (LCs) and turn them into activated APCs. The functional and phenotypic maturation of LCs and the decrease in number of LCs occur in the HPV-infected epidermis and disturb antigen presentation to T-cells [90–93]. The accumulation of tolerogenic APCs in the microenvironment can be the result of HPV affecting the extent of the CD40 signaling in the infected cells and consequently the production of cytokines and pro-inflammatory signals [94, 95]. HPV interferes with the production of cytokines and suppresses the antigen-presenting pathway, delaying the activation of the adaptive immune system. In adaptive immunity to HPV and escape mechanism, memory B-cells may release HPV capsid type-specific antibodies that can opsonize the virus and protect against subsequent infection with the same HPV type. In Ayer natural infection with HPV, the serum-neutralizing antibody levels are low as the infection is located intraepithelially. Seroconversion is generally detected within 18-month Ayer infection, but the level of Ig antibodies directed against the viral HPV capsids L1 and L2 is low or nonexistent in 30–50% of the patients [96, 97]. Control of HPV is achieved by activation of the HPV-specific interferon-a (IFN-a)-producing CD4+ and CD8+ type 1 T-cell responses to ER 22. The viral protein E2, E6, and E7 responses have been studied and were detected in the peripheral blood mononuclear cells (PBMCs) of healthy, HPV-negative but exposed subjects and in women with regression of their HPV-associated cervical lesions. In the majority of these women, circulating proliferating IFN-a- and IL-5-producing T-cells against E2, E6, and E7 were detected [98, 99]. It has been shown that the infiltration of low-grade squamous intraepithelial lesions by CD8+ cytotoxic cells is related with regression of the lesions, whereas the number of CTLs is substantially lower in patients with persistent low-grade cervical lesions [99, 100]. In patients with persistent HPV infection, this type of immunity is weak, and E6 and E7 are not detectable in the blood [101–105]. At the site of progressive high-grade squamous intraepithelial lesions, the number of infiltrating CD4+ and CD8+ T-cells is reduced and loses their ability to produce IFN-a. [100, 106]. Downregulation of HLA class I and class II molecules on HPV-transformed cells makes the infected cells less visible to the adaptive immune system and evades host immunity. This was shown in patients with cervical dysplasia where allelic loss of HLA-B44 expression showed progression of the lesions, while no downregulation was seen in nonprogressive lesions [107]. These data are consistent with the loss of HLA class I and HLA-A expression in cervical carcinomas [108, 109]. Nonclassical HLA types HLA-G, HLA-E, and MHC class I chain-related molecule A (MICA) are addressed to induce the pertinacity of HPV infections and lesions, as the expression of HLA-G and HLA-E is associated with progression of cervical intraepithelial

neoplasias to invasive squamous cell carcinoma [110, 111], and low expression of MICA is associated with impaired survival in patients with cervical tumors [109]. The expression of 23x cells and CTLs such as inhibitory molecules may result in suppression of the effector function of T-cells and may counteract migration of these cells to the infected lesions. This was demonstrated in different studies which showed that activated T-cells express inhibitory molecules such as cytotoxic T-lymphocyte antigen 4 (CTLA-4), program death 1 (PD-1), and T-cell immunoglobulin mucin-3 (TIM-3). Upon interaction with their ligands (CTLA-4 ligand, PD-ligand 1 and/or PD-ligand 2, and galectin-9), induction of apoptosis of x1 cells and inhibition of functional CTLs and x1 cells occur [112–114]. Also, tumor-associated (M2) macrophages and Tregs are attracted to the tumor site, where they form an immunosuppressive environment [115]. In high-grade lesions, the proliferation and function of effector T-cells are suppressed by Tregs, and it was shown that the ratio of tumor-infiltrating CD4+/CD8+ T-cells and the presence of Tregs in tumors are strongly associated with the prognosis and survival of patients with cervical cancer [109, 115, 116]. It was demonstrated that a strong intraepithelial infiltration of M1 macrophages was associated with a large influx of intraepithelial T lymphocytes, improving disease-specific survival [117]. Vaccination to prevent HPV infection and subsequently preclude HP-related disease is a valid strategy. Prophylactic vaccines aim to prevent an HPV infection by antibodies or humoral immune responses. These prophylactic HPV vaccines have no therapeutic effects as they do not increase viral clearance in subjects already infected with HPV [118]. For patients with progressive disease, multiple therapeutic immunotherapeutic modalities have been developed, of which therapeutic vaccination, non-specific immune stimulation with cytokines and antibodies, and adoptive cell therapy (ACT) are best known. Monoclonal antibodies directly mitigate the tumor-induced immunosuppressive conditions. The blockade of immune inhibitory pathways by targeting CTLA-4 (ipilimumab) and PD-1/PDL-1 (nivolumab) has demonstrated to be successful in preclinical studies and melanoma patients [119–122]. For the treatment of virus-induced malignancies and cancer, various therapeutic immunotherapies have been investigated with the goal to induce notable cell-mediated immunity [123]. In general, specificity is required to prevent destruction of healthy host tissue, and memory is required to prevent recurrences of primary tumors. A study focused on immunotherapy employed reinforcement of antigen-specific T lymphocytes [124]. A model has been proposed which took into account that transforming infection by HPV contributes to deregulation of the DNA methylation machinery, which, upon selection, may give rise to DNA methylation-mediated silencing of tumor suppressor genes [125] (Fig. 7.2).

Immunological Treatment Approaches for Premalignant Lesions

Several studies have shown increased immune activity, in premalignant lesions; however studies to determine the feasibility of immunotherapeutic approaches to treat lesions or to prevent their reoccurrence or progression to cancer have been few.

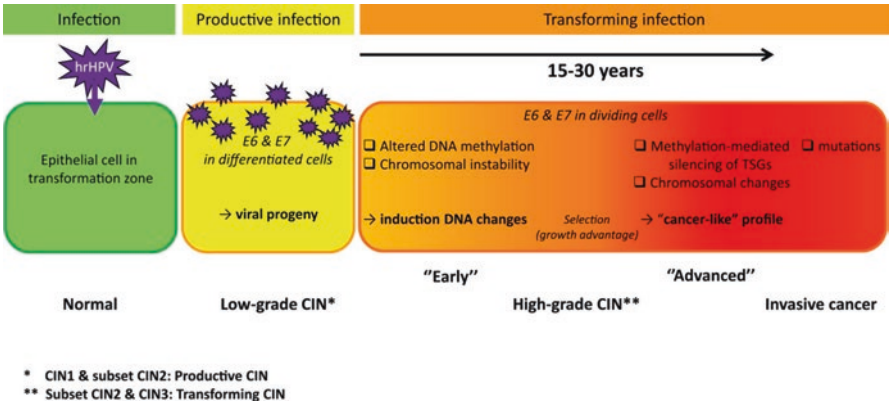

Fig. 7.2 Schematic representation of HPV-mediated cervical carcinogenesis. Progression of a high-grade CIN lesion, characterized by viral oncogene expression in dividing cells (i.e., a transforming infection), to invasive cancer results from the accumulation of DNA changes induced by HPV. High-grade CIN represents a heterogeneous stage of disease with varying duration of existence (up to 30 years). "Advanced" lesions show a cancer-like profile including hypermethylation of tumor suppressor genes and specific chromosomal alterations. Complementary somatic mutations only become detectable at the stage of invasive cancer. *CIN* cervical intraepithelial neoplasia, *TSG* tumor suppressor gene. (From Wilting and Steenbergen [237], with permission)

Squamous dysplasias have been shown to express some of the same tumor antigens as digestive tract carcinomas, namely, esophageal squamous cell carcinoma [126]. Similarly, tumor antigens were noted to be expressed by premalignant oral lesion of patients as are seen on head and neck squamous cell carcinomas [127]. Sharing of tumor antigens between premalignant oral lesions of a carcinogen-induced tongue lesion mouse model and the tongue cancers that developed from these lesions was also noted [128]. Some studies that have utilized immunotherapy for treatment of premalignant lesions and to prevent their progression to cancer have had varied results. Topical application of the agents, imiquimod and diclofenac, stimulates cytokine production and can trigger regression of premalignant skin actinic keratosis lesions [129, 130]. It was demonstrated that administration of selective inhibitors of cyclooxygenase-2 (COX-2) to rats diminishes the carcinogen-induced inflammatory NF-kB signaling pathways and slows the development of colonic tumors [131]. Also, administration of the select COX-2 inhibitor celecoxib to a mouse model of helicobacter-associated precancerous lesions tempered the immune inhibitory effects of PGE2 on expression of the Th1 cytokine IFN-γ and, consequently, accelerated the development of the premalignant lesions [132]. In a population-based, case-controlled study on the effectiveness of nonsteroidal anti-inflammatory drugs in subjects with Barrett's esophagus, whose progression to esophageal adenocarcinoma has been strongly shown to be inflammation-associated, no protective effects of the anti-inflammatory treatment on the incidence of cancer development were shown [133]. Treatment with anti-inflammatory compounds was found not to diminish the development of Barrett's esophagus in subjects with gastroesophageal

reflux disease [134]. There have been varied results of analyses of the effectiveness of nonsteroidal anti-inflammatory compounds and aspirin on the development of cancer in subjects with Barrett's esophagus [135]. A vaccination study in which patients with low-grade premalignant cervical abnormalities were vaccinated with a HPV16 synthetic long-peptide vaccine representing the E6 and E7 oncoprotein sequences showed HPV16-specific IFN-γ T-cell responses [136]. In another study using a mouse HPV tumor model to assess both immunological and clinical responses, peptide mixtures of the HPV E7 oncogene were shown to stimulate both antibody and cellular immune responses reactive to HPV constructs and to limit progression to malignancy [137]. Administration of a premalignant lesion-pulsed dendritic cell vaccine increased Th1 and Th17 immune reactivities and slowed progression to cancer [138]. In addition, insulin-like growth factor (IGF)-binding protein 2 and IGF receptor-I were used to test a vaccine consisting of peptides derived from these proteins in a TgMMTV-neu mouse model [138].

Targeted Immunotherapy of High-Grade Uterine Cervical Intraepithelial Neoplasia

Cervical cancer is the third most common cancer in women and the fifth most common overall cancer worldwide as age-standardized incidence rate in both sexes combined [139, 140]. The prime causal factor of the disease is a persistent infection with high-risk human papillomavirus (HPV), with individuals failing to mount adequate immune response against the virus. The high-risk HPV genome encodes three oncoproteins, E5, E6, and E7; the last two oncoproteins are constitutively expressed in high-grade lesions and cancer. These are required for the onset and maintenance of the malignant phenotype. About 170 HPV genotypes have been identified, and 40 can infect the anogenital area: the uterine cervix, vulva, vaginal wall, penis, and anus. HPVs are classified as high-risk types, commonly associated with cancer, and low-risk types, mostly identified in condyloma acuminatum. The International Agency for Research on Cancer (IARC) conducted a study on over 30,000 cervical cancers that showed HPV-16, HPV-18, HPV-58, HPV-33, HPV-45, HPV-31, HPV-52, HPV-35, HPV-59, HPV-39, HPV-51, and HPV-56 to be the most common types associated with invasive cervical cancer with HPV-16 accounting for over 50% and HPV-16 and HPV-18 for >70% worldwide [141]. Epidemiological data report that HPV infection occurs at least once during lifespan in about 75% of US women [142], and natural history shows that most HPV infections resolve spontaneously, while in some women, infection persists and progresses to cervical cancer. The incidence of high-grade cervical intraepithelial neoplasia 3 (CIN 3) is about one to two per ten females with low-grade CIN, and without treatment, about one third progresses to cervical cancer [143, 144]. Studies in HIV women or in patients treated with immunosuppressive agents reported an increased incidence of CIN lesions, suggesting an important role of cell-mediated immune response against HPV antigens [145, 146]. The role of systemic and local mucosal immune responses to HPV antigens is controversial. Some studies suggest a positive association

between systemic cell-mediated immune responses and the regression of CIN [147]. Moreover, antibody responses to the major viral capsid protein, L1, can be detected by about 6 months after infection and may be observed up to 5 years later in women who have been cleared from infection. Type-specific L1 antibody responses have also been detected in persistent disease and cancer in about half of the patients [148, 149]. The number of escape factors may affect the natural immune response against HPV proteins, together with the loss of correct signals from immune system to activate adaptive immune system. Indeed, optimal activation of adaptive immunity and generation of specific CD4 T-helper 1 type immunity supporting development of CD8 cytotoxic T-cells against viral early proteins, like E2, E6, and E7, are critical for virus clearance in basal epithelial cells. T-helper cells also support optimal activation of B-cells, with secreting HPV capsid type-specific neutralizing antibodies, which can protect against subsequent infections at mucosal and systemic levels [101]. Spontaneous regression occurs in lesions infiltrated by CD4+ and cytotoxic CD8+ T-cells, and it is also associated with circulating HPV early antigen-specific CD4+ and CD8+ T-cells [150–153]. The three oncogenes of the virus, E5, E6, and E7, play a notable role in immune evasion. The E5 protein [154] appears to facilitate the virus-induced immune escape by downregulating MHC/HLA class I and II [155, 156] and inducing a reduction in recognizing CD8+ T-cells [157]. This downregulation does not affect the HLA molecules (HLA-C/E) [158, 159]. Also, it has been shown that E5 selectively inhibits surface expression of HLA-A and HLA-B [155]. E6 and E7 still play an essential role: (i) high-risk E6 reduces the surface expression of CDH1 by epithelial cells; (ii) E6 and E7 inhibit the transcription of Toll-like receptor (TLR) 9, necessary to activate antigen-presenting cells as part of innate immune response; (iii) E7 reduces expression of transporter associated with antigen processing 1 (TAP1), a component of the presentation and processing pathway; and (iv) high-risk HPVs downregulates the expression of pro-inflammatory cytokines [160]. In addition, therapeutic T-cell effector mechanisms are limited due to the following: changes in local immunity, the production of cytokines such as interleukin (IL)-10, and increased number of regulatory T-cells (Tregs) and to immunosuppressive myeloid cells. Moreover, frequent mutational events in cancer include HLA loss of expression, with subsequent escape of tumor cells [161, 162]. To summarize, HPV-related tumors usually present MHC class I downregulation, impaired antigen-processing ability, avoidance of T-cell-mediated killing, increased immunosuppression due to Treg infiltration, and secretion of immunosuppressive cytokines [163]. These are obstacles faced when achieving a valid immunotherapy against HPV-related pathologies where a number of different strategies have been developed to overcome them including adjuvants. Certain adjuvants have recently been demonstrated to be able to induce cellular immunity which are summarized in Table 7.1 according to their mechanism of action [164].

Immunity can be utilized in a therapeutic setting in two ways: first, by using specific natural or synthetic antibodies against defined targets or, second, by inducing an immune response in the organism against specific antigens (preventive and therapeutic vaccines). Particularly, HPV-induced lesions and cancer viral antigens and/or virus-induced host antigens can be targeted by these approaches. Indeed,

Table 7.1 List of adjuvants by their dominant mechanism of action

Antigen delivery systems	Immunopotentiators
Electroporation	Alternative pathogen-associated molecular patterns (PAMPs), e.g., cholera enterotoxin, liquenase
Gene gun	Heat-shock proteins
Liposomes	Lysosome and endocellular reticulum (ER)-targeting agents
Virosomes™	Saponins (Quils, QS-21)
ISCOMS®	TLRs agonists, e.g., imiquimod, oligonucleotides (CpG, etc.), double-stranded RNA (dsRNA)
Micro/nanoparticles, e.g., microparticles of poly(lactide-co-glycolide) (PLG)	Cytokines and chemokines, e.g., IL2, IL12, and GM-CSF
Emulsions, e.g., MF59, Montanides	Treg inactivators, e.g., anti-apoptotic molecules, low-dose cyclophosphamide, antibodies anti-CD 25, anti-CTLA, anti-IL10, or anti-PDL-1
Viruslike particles and viral/bacterial vectors	Monophosphoryl lipid A (MPL) and synthetic derivatives
	Muramyl dipeptide (MDP) and derivatives

From Vici et al. [165], with permission

once a patient is infected with HPV, there is no effective way to cure persistent HPV infection which is the first step toward the development of precancerous lesions. It was estimated that with mass vaccination through highly effective preventive quadrivalent or bivalent HPV vaccines [165–169], it will take about 20 years or more before the prevalence of cervical cancer significantly decreases. As existing treatments [170–172] are partially effective in premalignant and malignant lesions, and invalid in persistent infections, immune therapies may offer a valid therapeutic modality. Table 7.2 focuses on the clinical trials of already established viral infections causing premalignant lesions of the uterine cervix [165].

The following are the therapeutic modalities of developing immunotherapeutic agents for premalignant uterine cervical lesions.

Therapeutic Antibodies

Intracellular antibodies (intrabodies) to inhibit protein function are valid pathways for the treatment of human diseases. This modality is effective and specific as it combats intracellular parasites like HPV viruses. Infected cells and transformed cells require the continuing of E6 and E7 oncogenes. This has been demonstrated in Hela cells, derived from an HPV-associated malignancy [173, 174]. Intrabodies against the E6 [175] and E7 [176] of HPV have been produced and proved effective in in vitro cancer cell models. An intrabody against the E7 of HPV-16 has been shown to inhibit tumor growth in animal models [177]. Intrabodies are thought to be useful inhibitors of viral protein-protein interactions and appropriate for the treatment of HPV-associated diseases. The utilization of monoclonal antibodies against membrane-expressed antigens may be induced by the HPV, i.e., epidermal growth

Table 7.2 Clinical trials for HPV-associated pre-neoplastic cervical lesions

Vaccine	Antigen(s)	Phase	Lesions
ADXS11–001:	HPV-16 E7	II	CIN 2/3
Lm secreting fusion/LLO-HPV-16 E7 protein (Lm-LLO-E7)	HPV-16 and HPV-18 E7	I/II	High-risk HPV infections before CIN appearance
ProCervix: adenylate cyclase protein vector delivering HPV16 and HPV18 E7 antigens			
MVA E2: recombinant modified vaccinia Ankara (MVA) encoding E2 from BPV	Bovine papillomavirus E2	I/II	CIN1–3
		II	High-grade CIN
TG4001/R3484:	HPV-16 E6/E7	IIa	CIN 2/3
Recombinant MVA expressing E6–E7of HPV-16 and IL-2		IIb	
Peptides: HPV E7 (aa 12–20) plus E7 lipopeptide (PADRE helper peptide, linker peptide, and E7 peptide, aa 86–93) and Montanide ISA-51 adjuvant	HPV-16 E7	I	High-grade CIN and
			HSIL
HPV-16 E6/E7 fusion protein plus ISCOMATRIX adjuvant	HPV-16 E6 and E7	I	CIN 1–3, HPV-associated AIN in HIV-positive male
PD-E7: Modified HPV-16 E7/Hib protein D fusion protein and AS02B adjuvant	HPV-16 E7	I/II	CIN 1, CIN 3
SGN-00101: HPV-16 E7/*M. bovis*, Hsp65 fusion protein		II	ASCUS and LSIL, high-grade CIN
SGN-00101 in poly-ICLC adjuvant	HPV-16 E7	I	CIN 1–3
ZYC101: Recombinant HPV-16 E7 DNA plasmid encapsulated in poly-microparticles	HPV-16 E7	I	CIN 2/3
ZYC101a: Recombinant HPV-16 and HPV-18 E6–E7 DNA plasmid encapsulated in poly-microparticles	HPV-16 and HPV-18 E6 and E7	II/III	High-grade CIN
pNGVL4a-Sig/E7/Hsp70: DNA plasmid expressing mutated HPV-16 E7 fused to Sig and Hsp70	HPV-16 E7	I	CIN 2/3
pNGVL4a-CRT/E7: DNA plasmid expressing mutated HPV-16 E7 fused to calreticulin	HPV-16 E7	I	CIN 2/3
VGX-3100: DNA plasmid expressing HPV-16 and HPV-18 E6 and E7 proteins	HPV-16 and HPV-18 E6 and E7	I	CIN 2/3 (after surgery or fourth dose)
		II	CIN 2/3
TA-CIN/TA-HPV prime/boost	HPV-16 and HPV-18 E6 and E7 and HPV-16 L2	II	CIN 2/3
TA-HPV/TA-CIN prime/boost	HPV-16 and HPV-18 E6 and E7 and HPV-	II	CIN 2/3

(continued)

Table 7.2 (continued)

Vaccine	Antigen(s)	Phase	Lesions
pNGVL4a-Sig/E7 /Hsp70 and TA-HPV prime/boost plus TLR agonist imiquimod	HPV-16 and HPV-18 E6 and E7	II	CIN 2/3

From Vici et al. [165], with permission

factor receptor (EGFR). Monoclonal antibodies anti EGFR are currently clinically utilized [170]. Other membrane-associated antigens can be found in transformed cervical cells and may be targeted by monoclonal antibodies. Adecatumumab (MT201), a humanized monoclonal antibody targeting epithelial cell adhesion molecules, is an example of these antibodies. It has shown some activity in cervical cancer cell lines overexpressing epithelial cell adhesion molecule (EpCAM) [178].

Therapeutic Vaccines

Therapeutic vaccines aim to kill or reduce infected cells by stimulating cytotoxic T-cells against target infected cells and upregulating MHC class I expression. Vaccine-mediated immune strategies have two stages of the oncogenic infection: firstly, infection and then, secondly, the established infection. By eliciting neutralizing antibody responses, the prophylactic vaccines challenge the first infection by inhibiting the HPV to bind to the cell or the early phases of viral entry. The therapeutic vaccines could be tailored based on the presence of episomal replicating virus or integrated viral sequences. In the first case, the vaccine targets early proteins; in the second case, it targets E6–E7 proteins [179]. Effective immunotherapy administered before tumor challenge includes an antigen-specific component, whereas an effective immunotherapy after tumor challenge can be achieved through the enhancement of either innate or adaptive immunity. Immunotherapy in patients with HPV-associated premalignancy is more effective than in cancer patients, as the impaired antigen presentation by cervical cancer cells due to mutations in MHC and TAP genes may render the immunotherapy less effective. However, there are potential immune-evasive mechanisms that are attributed to the HPV infection [180]. Examples of those therapeutic vaccines are as follows:

Dendritic Cell (DC)-Based Vaccines

The immune response to infection causes inflammatory responses that trigger innate effector cells, such as NK and NKT cells. This inflammatory response, driving the innate immunity, is initiated through pathogen-associated molecular pattern (PAMP) sensors including TLRs 1–9. These receptors in response to specific bacterial or viral components activate APCs via the transcription factor nuclear factor-KB (NF-KB). Also, infection may alter the local metabolic and cellular microenvironment activating danger-associated molecular pattern (DAMP) sensors, specially nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), inducing maturation and releasing members of the IL-1 family. The produced IL-1b and

IL-18 mediate repair responses such as angiogenesis and, via upregulation of cytokines and chemokines, induce the recruitment of inflammatory cells to the site of infection. The slow clearance of HPV infection and weak immune responses to viral proteins are consequences of the nonlytic nature of HPV infection and a consequent delay in induction of PAMP- and DAMP-induced inflammatory responses through TLRs and the inflammasomes. In the absence of inflammation, IL-10 production by Th cells and mast cells, IFN-gamma production by CD-1d-activated NKT cells, and increased TGF-beta occur inducing negative signals that change the state of the APC by altering co-stimulatory molecule expression, thus inhibiting induction of cytotoxic effector T-cells. Consequently, a therapy aimed to reactivate these APCs could be a valid tool for clinical intervention. DCs are the most potent APC as they express high levels of MHC and co-stimulatory molecules. A variety of methods have been established for generating DCs, loading them with tumor antigens, and administering them to patients. Provenge, a DC vaccine incorporating prostatic acid phosphatase, has been studied in patients with advanced prostate cancer [181, 182]. In a study, autologous DCs were pulsed with HPV-16 or HPV-18 E7 recombinant proteins, and E7-specific CD8+ T-cell responses were observed in 4 out of 11 late-stage cervical cancer patients [183]. In another study, stage IB or IIA cervical cancer patients were vaccinated with autologous DC pulsed with recombinant HPV-16/HPV-18 E7 antigens and keyhole limpet hemocyanin 1 (KLH1). This vaccine generated E7-specific T-cell responses in eight out of ten patients and antibody responses in all patients [184].

Nucleic Acid-Based Vaccines

DNA vaccines have been used to elicit antigen-specific immune responses. They have several advantages; mainly naked DNA is relatively safe, stable, cost-efficient, and able to sustain reasonable levels of antigen expression within cells. DNA-based plasmid vectors remain stable in a wide range of conditions over a long time, and they can be delivered with slight risk to individuals who are immunosuppressed. Also, they can be repeatedly administered with similar efficacy. Many strategies have been employed to produce an efficient delivery of targeted antigen-to-antigen-presenting cells (APC) such as dendritic cells (DCs), an enhancement of antigen processing and presentation in DCs, and an augmentation of DC and T-cell interaction [185]. It has been reported that the fusion of the E7 gene of HPV-16 with a plant virus coat protein produced a strong antitumor activity in a mouse model activating both CD4+ and CD8+ T-cells [186–188] and a fusion of E7 gene to a gene encoding a mutated form of the immunotoxin from *Saponaria officinalis*, the saporin [187]. A dose-escalation trial of plasmid DNA encoding a transgene that produced E7 linked to Hsp70 showed a limited efficacy at the highest dose, with low induction of responses in the IFN-gamma ELISPOT assay and a resolution rate of 33% [188]. A plasmid DNA encoding a 13-amino acid sequence of E7 encapsulated in biodegradable poly(D, L-lactide-co-glycolide) microparticles was utilized to develop the ZYC101 vaccine expressing HPV-16 E7 HLA-A2-restricted peptide. Another two different phase I clinical trials examining the potential treatment of patients with anaplasia and with high-grade CIN, respectively, showed a high number of

immunological responses, circulating HPV-specific T-cells and histological regression/improvement in 1/3 of the patients [189, 190]. Version ZYC101a that includes the HPV-encoding sequences of HPV-16 E7, the regions encoding segments of HPV-16 and HPV-18 E6 and E7 viral proteins, has reached phase II/III clinical trials involving patients with high-grade CIN. In a population of women younger than 25 years, CIN resolution was significantly higher in the ZYC101a groups compared to placebo [191]. In addition, it was evaluated in the treatment of patients with CIN 2/3 where half of 21 patients receiving the vaccine showed HPV-16-/HPV-18-specific T-cell responses, but only 6 patients recovered from high-grade CIN [192]. The methodologies for production and delivery of HPV therapeutic vaccines are shown in Fig. 7.3 [193].

VGX-3100, a DNA vaccine incorporating plasmids targeting HPV-16 and HPV-18 E6 and E7 proteins, was utilized in a phase I clinical trial; 78% of the VGX-3100-vaccinated high-grade CIN subjects showed T-cell and antibody responses [194]. Other DNA vaccines have also been associated with other adjuvating treatments, namely, the TLR7 agonist, imiquimod, promoting the activation of antigen-presenting cells and leading to the production of cytokines IFN-alpha, IL-6, and TNF-alpha [195] which was shown to be active in mouse models [196]. Notably, the imiquimod treatment affected the tumor microenvironment by reducing the number of myeloid-derived suppressor cells that have an immunosuppressive role and increasing natural killer (NK) and NKT cells that may play a role in tumor volume reduction. Moreover, the use of RNA replicons is a potentially valid strategy for HPV vaccination. RNA replicons are naked RNA molecules derived from alphaviruses, such as Sindbis virus [194, 195], Semliki Forest virus [196, 197], and Venezuelan equine encephalitis (VEE) virus [198]. These RNA vaccines are self-replicating and self-limiting and may be administered as either RNA or DNA, which is then transcribed into RNA replicons. RNA replicon-based vectors can replicate in a wide range of cell types and can be used to produce sustained levels of antigen expression in cells, making them more immunogenic than conventional DNA vaccines. Notably, RNA replicons are less stable than DNA. To combine the benefits of DNA and RNA replicon, DNA-launched RNA replicon was utilized for HPV vaccine development in preclinical models [199, 200]. This DNA-launched RNA replicon is transcribed into RNA within the transfected cell and provides an efficient way to express tumor antigen, but it induced cellular apoptosis. Another replicon system is derived from the flavivirus Kunjin (KUN) which has been utilized [201]. The new generation of KUN replicon vectors did not induce cellular apoptosis, and it elicited specific T-cell responses [202]. Another mRNA-based vaccine is the RNActive® vaccine platform which is based on a more stable modified mRNA sequence with increased immunogenicity by complexation with protamine. This mRNA vaccine exploits both the antigenic and the adjuvant properties of mRNAs to activate the adaptive and innate immune system.

Live Vector-Based Vaccines

Bacteria, such as *Listeria monocytogenes* (LM), *Lactococcus lactis*, *Lactobacillus casei*, *Salmonella*, and bacillus Calmette-Guerin, and several viral vectors, including vaccinia virus (VV), adenovirus, adeno-associated virus, alphavirus, and its

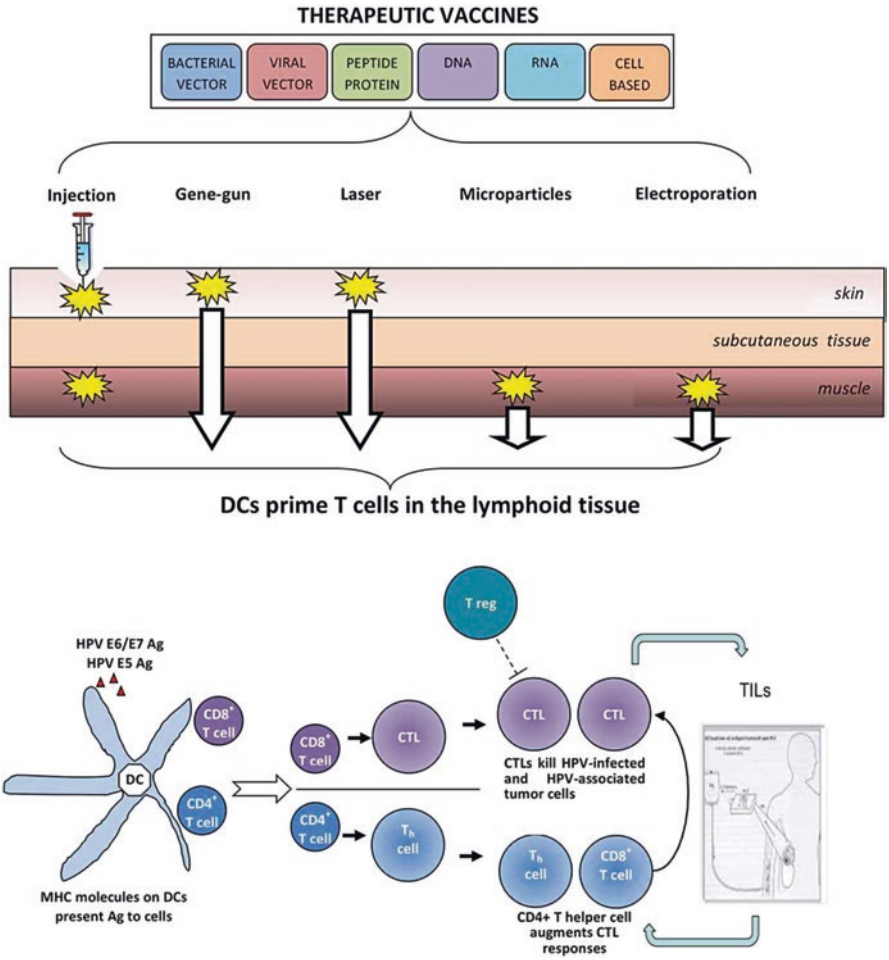

Fig. 7.3 Methodologies for production and delivery of HPV therapeutic vaccines and their immunological activity. Abbreviations: *Ag* antigen, *DCs* dendritic cells, *Treg* regulatory T-cell, *Th* T-helper cell. (From Vici et al. [194], with permission)

derivative vectors, have been used to deliver genes to elicit antigen-specific immunotherapy.[203–208] LM has emerged as a promising vector, as it is able to induce both CD8+ and CD4+ immune responses, to elicit regression of established tumors, and to overcome central tolerance by expanding low-avidity CD8+ T-cells specific for E7 [209, 210]. DXS11–001 a live, attenuated LM bacterial vector secreting HPV-16 E7 fused to listeriolysin O (LLO) was utilized in clinical trials [211, 212]. Several trials are ongoing involving women with persistent or recurrent cervical carcinoma (NCT01266460), with CIN 2/3 with surgical indication (NCT01116245) [213], and patients (including male) with HPV-associated oropharyngeal cancer (NCT01598792). Viral vectors are employed for the expression of HPV antigens, like adenoviruses [214], alphaviruses [215–217], and VV [218–220]. VV vaccines

were the first viral vectors employed in clinical trials on therapeutic vaccines against HPV-associated cancer [221]. Recently avipox viruses have been developed as novel vectors for the development of vaccines. Avipox viruses have been shown to inhibit the growth of HPV16 E7-expressing tumor in C57 Bl6 mice with a HPV16 E7 DNA-prime/Fowlpox HPV16 E7-boost schedule [222]. Several VV vaccines have been employed in clinical trials to deliver genes and antigens of interest efficiently. Phase I/II clinical trials in patients with vulvar, vaginal, and early- and late -stage cervical cancer are conducted with a vaccinia vector encoding HPV-16 and HPV-18 E6 and E7 antigen (TA-HPV) recombinant VV [223–225]. In a phase II clinical trial, 29 patients with stage I or II cervical cancer were vaccinated twice via scarification with TA-HPV; induction of CTL responses were detected in a number of patients in the form of target cell lysis by isolated peripheral bone marrow cells (PBMCs) [226]. In another study, a recombinant VV expressing E6 and E7 antigen together with IL-2 (TG4001/R3484) was administered to CIN 2/3 patients. Ten patients (48%) were evaluated as clinical responders at month 6. At month 12, 7 out of 8 patients without conization reported neither suspicion of CIN 2/3 relapse nor HPV-16 infection [227]. Another phase IIb trial on patients with HPV-related CIN 2/3 lesions demonstrated the activity of vaccine in monotherapy [228]. A recombinant modified vaccinia Ankara vector was also utilized to express bovine papillomavirus E2 (MVA-E2). E2 is a transcriptional repressor of E6 and E7 oncogenes. There is no evidence for E2 expression direct contribution to the therapeutic effect seen in patients with CIN [229, 230] and genital wart [231] response. Synthetic viral vectors like viruslike particle (VLP) can be utilized as they have the capacity for compacting DNA and targeting specific cell receptors. The same technology used for producing anti-HPV prophylactic vaccines was employed for producing chimeric VLPs. An L1–E7 fusion protein has been shown to self-assemble into chimeric VLPs (CVLP) that can induce E7-specific cellular immunity in mice [232]. A randomized, double-blind, placebo-controlled clinical trial has been conducted in CIN 2/3 patients with CVLP. Antibodies with high titers against HPV-16 L1 and low titers against HPV-16 E7 and cellular immune responses against both proteins were induced. A histological improvement to CIN I or normal histology was observed in 39% of the patients [233].

Plant-Derived/Produced Vaccines

Plant molecular biotechnology includes the production of protein biopharmaceuticals such as enzymes, hormones, antibodies, and vaccine antigens in plant systems. The plant platforms present several drawbacks: time-consuming in generating stable transgenic lines, nonhomogeneous protein production in different tissues, impact of pests and diseases, and growth in non-sterile conditions [185–187]. Plant production of prophylactic and therapeutic HPV vaccines is proven, with evidence of efficacy in animals. There are data showing that an adjuvant-like effect was obtained in immunizations with crude tobacco plant extracts containing the E7 protein of HPV-16 [215, 216]. The recombinant plant-derived vaccines without adjuvants were able to elicit also a protective Th1 cell response in mice. A similar adjuvating activity was seen in another tobacco plant-produced fusion protein of the

HPV-16 E7; this preparation was able to induce a specific CD8+ T stimulation that elicited a therapeutic effect on experimental tumor models [188, 189]. The possibility to produce E7 with high immunological activity in microalgae opens the way to producing antigens at affordable price, retaining the adjuvating activity of these plant-derived antigens [217]. An FDA-approved clinical trial for non-Hodgkin's lymphoma with plant-produced single-chain variable fragment (scFv) was able to establish the safety and immunogenicity of plant-made human vaccines [218, 219]; this could be a feasible approach for human anticancer therapies.

Protein-/Peptide-Based Vaccines

There are several protein-/peptide-based vaccines undergoing clinical evaluation. A major limitation to peptide-based vaccines is the HLA restriction that can be overcome by whole protein-based vaccines, which harbor multiple immunogenic epitopes, binding various allelic HLA molecules. A majority of studies were focused on the co-administration of adjuvant immune-enhancing agents such as chemokines, cytokines, and co-stimulatory molecules to enhance the potency of the vaccine. Particularly, saponin-based [152] or liposome-based (LPD) formulations [153] or TLR agonists [154] were employed as adjuvants for protein vaccines. Recently, the fusion of the beta-1,3-1,4-glucanase (LicKM) of *Clostridium thermocellum* bacterial protein to the HPV E7 protein produced an antigen with strong intrinsic adjuvating activity, indicating that it may lead to elicit some functions [155, 156]. Many other fusion proteins were reported to elicit some adjuvating activities such as *Mycobacteria*-derived heat-shock proteins (Hsp) [157, 158], truncated *Pseudomonas aeruginosa* exotoxin A [159], *Bordetella pertussis* adenylate cyclase [160], and the cell-penetrating peptide *Limulus polyphemus* protein [161]. TLR agonists have been explored as adjuvants for peptide-based HPV vaccines because of their capability to activate both innate and adaptive immunities. Vaccines consisting in CTL and/or TH epitope adjuvated with TLR 9 [162]; TLR4 [163] and/or TLR3 [164] agonists demonstrated their efficacy in mouse models. This activity was demonstrated also by utilizing a CTL epitope fused to a T-helper epitope, pan-DR epitope (PADRE) [165]. Adjuvants targeting dendritic cells are useful in peptide-based vaccines. A strategy based on the administration of co-stimulatory anti-CD40 monoclonal, TLR agonist polyinosinic-polycytidylic acid [poly(I:C)] and CD8+ T-cell epitope HPV-16 E7 (aa49–57) was able to induce tumor clearance in two HPV-induced murine cancer models [166]. SGN-00101 vaccine, a fusion protein consisting of Hsp from *Mycobacterium bovis* and HPV-16 E7, has shown that it was able to induce regression of lesions in anal high-grade squamous intraepithelial lesions [167], recurrent respiratory papillomatosis [168], and CIN 2/3 [169–171]. Phase II clinical trial with TA-CIN, a fusion protein-based vaccine expressing HPV-16 L2-E6–E7-conjugated proteins, in conjunction with topical application of TLR agonist imiquimod showed high levels of CD4+ and CD8+ T-cells locally in patients with high-grade vulvar intraepithelial neoplasia (VIN) [172]. The PADRE universal T-helper peptide was utilized to increase the activity of CTL epitopes encoding HPV-16 E7 that was presented by HLA-A*0201. These vaccines failed to achieve a valid immune response in women with late-stage cervical cancer [168–170]. More promising results were

obtained in HLA-A2-positive patients with CIN/VIN 2/3 [176], where HPV E7 lipopeptide (aa 86–93)/PADRE was able to stimulate an immune response and led to complete regression of CIN lesions in 3 of 17 valuable patients. In resected cervical cancer patients, the use of immunization with 13 overlapping long peptides spanning the entire sequence of HPV-16 E6 and E7 mixed with Montanide ISA 51 clearly revealed immunization-driven IFN-gamma production in enzyme-linked immunospot (ELISPOT) assay after completing the protocol [176]. The same platform was tested in immunizing cervical cancer patients and showed that both CD4+ and CD8+ T-cell IFN-gamma responses were detected toward both antigens [178]. Significant increases in proliferative capacity were also noted in responding T-cells [178]. Phase II clinical trials of this vaccine in histologically confirmed HPV-16-positive high-grade VIN patients had a complete regression of their lesion after three or four vaccinations with HPV-16 E6/E7 overlapping peptide vaccine [179]. In non-responders to the vaccine, an increased number of HPV-16-specific CD4 + CD25 + Foxp3+ Treg cells were noted [180]. The presence of these Foxp3+ T-cells is linked to impaired immunity in malignancies. The efficacy of this vaccine was also shown in a phase II study that noted an increased number of HPV-16-specific T-cells in patients with HPV-16+ high squamous intraepithelial lesion (HSIL) [181].

Combinational Immunotherapy

Strategies aiming to alter local immunity have shown positive results; thus therapeutic HPV vaccine strategies have shifted toward combinatorial approaches with radiotherapy and chemotherapy. Low-dose radiation in combination with HPV vaccination was effective in the treatment of tumors in preclinical models [220]. Radiation therapy seems to be a useful method in stabilizing tumor cell growth when applied with immunotherapy by inducing apoptosis in tumor cells. A chemotherapeutic agent in combination with DNA-based vaccines was shown to be an effective HPV therapy in preclinical models [221, 222]. Low-dose cyclophosphamide produced positive effects in persistent low-risk HPV lesions [223]. A randomized study was carried out in 110 recurrent/refractory cervical cancer patients with cisplatin and different doses of HPV bacterial vector-based vaccine ADXS11–001, and results showed efficacy and manageable toxicity [224]. Other compounds affecting the immunological environment like COX-2 inhibitors, through the prevention of the production of prostaglandin E2 or antibodies to IL-6 [225] or IL-10 [226] or the TLR agonist imiquimod, could be a valid therapeutic agent. Imiquimod is currently in clinical use against warts stimulating local innate immunity and potentiating adaptive immune response by activating tissue antigen-presenting cells. Several studies with topical imiquimod have been reported with favorable results in vulvar intraepithelial neoplasia (VIN) lesions [227, 234]. Cytokine-based therapies in combination with HPV therapeutic vaccine showed promising results in preclinical models. Treatment with IL-12 gene, administered as gene therapy, as viral gene therapy, by adenovirus, and in combination with E6–E7 oncogenes,

determined tumor growth suppression [228, 229]. An anti-PD-1 antibody (CT-011) with Treg-cell depletion by low-dose cyclophosphamide (CPM), combined with HPV-16 E7 peptide vaccine, produced antigen-specific immune responses inducing complete regression of established tumors in a notable percentage of treated animals, with prolonging survival [230]. Expanded phase I clinical studies with anti-PD-1 and anti-PDL-1 showed objective clinical responses in renal cell carcinoma, melanoma, and non-small cell lung cancer and a relationship between tumor cell surface PD-L1 expression and objective responses to anti-PD1 therapy [231, 232]. In addition, a recent study showed that PD-1/PDL-1 pathway may create an "immune-privileged" site for initial viral infection in the tonsils and subsequent adaptive immune resistance once tumors are established suggesting a rationale for therapeutic blockade of this pathway in patients with HPV + oropharyngeal squamous cell carcinoma [233]. Other strategies utilize monoclonal antibodies such as ipilimumab. This antibody is a fully human monoclonal antibody against the cytotoxic T-lymphocyte antigen-4 (CTLA-4), an immune inhibitory molecule expressed in activated T-cells and in suppressor T-regulatory cells. The interaction between the monoclonal antibody and CTLA-4 blocks inhibitory signals and enhances T-cell activation, leading to increased antitumor responses [235].

Conclusion

Human cancer has a number of unique features. Immune infiltration into the tumor has been demonstrated, but tumor evasion and subversion of these immune defenses were noted. In the immunosuppressive environment of the tumor, achieving immune reactivity through immunotherapeutic approaches is difficult. There are a number of precancerous lesions that pose a high risk of developing into cancer. It is difficult to determine if the precancerous lesion environment would be less immune subversive than the one for cancer and would be better suited for immunotherapeutic treatment approaches. However, immunotherapy is a promising strategy for cancer treatment. In cervical cancer and its precursors, the use of therapeutic vaccines was associated with the regression of premalignant lesions and some clinical benefit in cancer patients. Current data suggest that vaccines for pre-neoplasia and cancer of the uterine cervix are valid therapeutic modalities. The improvement of all therapeutic strategies and the identification of their optimal combination open an efficient scenario in the treatment of uterine cervical cancer and its premalignant lesions. As the role of immunotherapy for the treatment of patients with precancerous lesions and uterine cervical cancer continues to evolve, further studies on immune cellular and molecular mechanisms of action and on preclinical models are needed to better understand immunological background and to explore the optimal integration among treatments and combination immunotherapies.

References

1. Scott AM, Wolchok JD, Old LJ. Antibody therapy of cancer. Nat Rev Cancer. 2012;12(4):278–87.
2. Perez SA, Karamouzis MV, Skarlos DV, Ardavanis A, Sotiriadou NN, Iliopoulou EG. CD4+CD25+ regulatory T-cell frequency in HER-2/neu (HER)-positive and HER-negative advanced-stage breast cancer patients. Clin Cancer Res. 2007;13(9):2714–21.
3. Park T, Choi CJ, Choi Y, Suh DC. Cost-effectiveness of cetuximab for colorectal cancer. Expert Rev Pharmacoecon Outcomes Res. 2016;16(6):667–77.
4. Gilbert MR, Pugh SL, Aldape K, Sorensen AG, Mikkelsen T, Penas-Prado M. NRG oncology RTOG 0625: a randomized phase II trial of bevacizumab with either irinotecan or dose-dense temozolomide in recurrent glioblastoma. J Neuro-Oncol. 2016;131:193–9.
5. Lee S, Margolin K. Cytokines in cancer immunotherapy. Cancers. 2011;3(4):3856–93.
6. U.S., Food and Drug Administration. Intron A. Label information. http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/103132s5191lbl.pdf. 6 Apr 1986.
7. U.S., Food and Drug Administration. Aldesleukin product approval information – licensing action. http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm080733.htm. 1 Sept 1998.
8. Scheid E, Major P, Bergeron A, Finn OJ, Salter RD, Eady R. Tn-MUC1 DC vaccination of rhesus macaques and a phase I/II trial of patients with non-metastatic castrate-resistant prostate cancer. Cancer Immunol Res. 2016;4(10):881–92.
9. Mittendorf EA, Ardavanis A, Litton JK, Shumway NM, Hale DF, Murray JL. Primary analysis of a prospective, randomized, single-blinded phase II trial evaluating the HER2 peptide GP2 vaccine in breast cancer patients to prevent recurrence. Oncotarget. 2016;7(40):66192–201.
10. Engelstein R, Merims S, Eisenberg G, Cohen J, Frank S, Hamburger T. Immune monitoring of patients treated with a whole-cell melanoma vaccine engineered to express 4-1BBL. J Immunother. 2016;39(8):321–8.
11. Courau T, Nehar-Belaid D, Florez L, Levacher B, Vazquez T, Brimaud F. TGF-β and VEGF cooperatively control the immunotolerant tumor environment and the efficacy of cancer immunotherapies. JCI Insight. 2016;1(9):e85974.
12. Rong L, Li R, Li S, Luo R. Immunosuppression of breast cancer cells mediated by transforming growth factor-β in exosomes from cancer cells. Oncol Lett. 2016;11(1):500–4.
13. Cui C, Feng H, Shi X, Wang Y, Feng Z, Liu J. Artesunate down-regulates immunosuppression from colorectal cancer Colon26 and RKO cells in vitro, by decreasing transforming growth factor β and interleukin-10. Int Immunopharmacol. 2015;27(1):110–21.
14. Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet. 2016;387(10030):1837–46.
15. Eberstal S, Sanden E, Fritzell S, Darabi A, Visse E, Siesjo P. Intratumoral COX-2 inhibition enhances GM-CSF immunotherapy against established mouse GL261 brain tumors. Int J Cancer. 2014;134(11):2748–53.
16. Mao Y, Poschke I, Wennerberg E, Pico de Coana Y, Egyhazi Brage S, Schultz I. Melanoma-educated $CD14^+$ cells acquire a myeloid-derived suppressor cell phenotype through COX-2-dependent mechanisms. Cancer Res. 2013;73(13):3877–87.
17. Wang X, Wang L, Mo Q, Dong Y, Wang G, Ji A. Changes of Th17/Treg cell and related cytokines in pancreatic cancer patients. Int J Clin Exp Pathol. 2015;8(5):5702–8.
18. Yu GT, Bu LL, Huang CF, Zhang WF, Chen WJ, Gutkind JS. PD-1 blockade attenuates immunosuppressive myeloid cells due to inhibition of CD47/SIRPα axis in HPV negative head and neck squamous cell carcinoma. Oncotarget. 2015;6(39):42067–80.
19. Walsh JE, Clark AM, Day TA, Gillespie MB, Young MR. 3, treatment to stimulate immune infiltration into head and neck squamous cell carcinoma. Hum Immunol. 2010;71:659–65.

20. Li T, Yi S, Liu W, Jia C, Wang G, Hua X. Colorectal carcinoma-derived fibroblasts modulate natural killer cell phenotype and antitumor cytotoxicity. Med Oncol. 2013;30(3):663.
21. Mulligan JK, Young MR. Tumors induce the formation of suppressor endothelial cells in vivo. Cancer Immunol Immunother. 2010;59(2):267–77.
22. Benard VB, Castle PE, Jenison SA, Hunt WC, Kim JJ, Cuzick J. Population-based incidence rates of cervical intraepithelial neoplasia in the human papillomavirus vaccine era. JAMA Oncol. 2016;6:833–7.
23. Prue G, Lawler M, Baker P, Warnakulasuriya S. Human papillomavirus (HPV): making the case for 'Immunisation for all'. Oral Dis. 2016;23:726–30.
24. Huber MA. Adjunctive diagnostic aids in oral cancer screening: an update. Tex Dent J. 2012;129(5):471–80.
25. Doubeni CA, Corley DA, Quinn VP, Jensen CD, Zauber AG, Goodman M. Effectiveness of screening colonoscopy in reducing the risk of death from right and left colon cancer: a large community-based study. Gut. 2016;67:291–8.
26. Rethman MP, Carpenter W, Cohen EE, Epstein J, Evans CA, Flaitz CM. Evidence-based clinical recommendations regarding screening for oral squamous cell carcinomas. Tex Dent J. 2012;129(5):491–507.
27. Saslow D, Solomon D, Lawson HW, Killackey M, Kulasingam SL, Cain J. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. Am J Clin Pathol. 2012;137(4):516–42.
28. Freeman A, Bridge JA, Maruthayanar P, Overgaard NH, Jung JW, Simpson F. Comparative immune phenotypic analysis of cutaneous squamous cell carcinoma and intraepidermal carcinoma in immune-competent individuals: proportional representation of CD8+ T-cells but not FoxP3+ regulatory T-cells is associated with disease stage. PLoS One. 2014;9(10):e110928.
29. Ohman J, Mowjood R, Larsson L, Kovacs A, Magnusson B, Kjeller G. T-cells in oral premalignant leukoplakia indicates prevention of cancer transformation. Anticancer Res. 2015;35(1):311–7.
30. Ohman J, Magnusson B, Telemo E, Jontell M, Hasseus B. Langerhans cells and T cells sense cell dysplasia in oral leukoplakias and oral squamous cell carcinomas – evidence for immunosurveillance. Scand J Immunol. 2012;76(1):39–48.
31. Woodford D, Johnson SD, De Costa A-MA, Young MRI. An inflammatory cytokine milieu is prominent in premalignant oral lesions, but subsides when lesions progress to squamous cell carcinoma. J Clin Cell Immunol. 2014;5(3):1–17.
32. Kavanagh ME, Conroy MJ, Clarke NE, Gilmartin NT, Feighery R. Impact of the inflammatory microenvironment on T-cell phenotype in the progression from reflux oesophagitis to Barrett oesophagus and oesophageal adenocarcinoma. Cancer Lett. 2016;370(1):117–24.
33. Miyashita T, Tajima H, Shah FA, Oshima M, Makino I, Nakagawara H. Impact of inflammation-metaplasia-adenocarcinoma sequence and inflammatory microenvironment in esophageal carcinogenesis using surgical rat models. Ann Surg Oncol. 2014;21(6):2012–9.
34. Garay J, Piazuelo MB, Majumdar S, Li L, Trillo-Tinoco J, Del Valle L. Helicobacter pylori, and in development of mucous metaplasia in mice. Cancer Lett. 2016;371(1):90–8.
35. Lian J, Ma L, Yang J, Xu L. Aberrant gene expression profile of unaffected colon mucosa from patients with unifocal colon polyp. Med Sci Monit. 2015;21:3935–40.
36. Ben-Horin S, Izhaki Z, Haj-Natur O, Segev S, Eliakim R, Avidan B. Rarity of adenomatous polyps in ulcerative colitis and its implications for colonic carcinogenesis. Endoscopy. 2016;48(3):215–22.
37. He Y, Zha J, Wang Y, Liu W, Yang X, Yu P. Tissue damage-associated "danger signals" influence T-cell responses that promote the progression of preneoplasia to cancer. Cancer Res. 2013;73(2):629–39.
38. Zhang B, Kwon OJ, Henry G, Malewska A, Wei X, Zhang L. Non-cell-autonomous regulation of prostate epithelial homeostasis by androgen receptor. Mol Cell. 2016;63(6):976–89.
39. Liou GY, Doppler H, Necela B, Edenfield B, Zhang L, Dawson DW. Mutant KRAS-induced expression of ICAM-1 in pancreatic acinar cells causes attraction of macrophages to expedite the formation of precancerous lesions. Cancer Discov. 2015;5(1):52–63.

40. De Costa AM, Schuyler CA, Walker DD, Young MR. Characterization of the evolution of immune phenotype during the development and progression of squamous cell carcinoma of the head and neck. Cancer Immunol Immunother. 2011;61(6):927–39.
41. Johnson SD, De Costa AM, Young MR. Effect of the premalignant and tumor microenvironment on immune cell cytokine production in head and neck cancer. Cancers. 2014;6(2):756–70.
42. Hardikar S, Onstad L, Song X, Wilson AM, Montine TJ, Kratz M. Cancer Epidemiol Biomark Prev. 2014;23(11):2393–403.
43. Juretic M, Cerovic R, Belusic-Gobic M, Brekalo Prso I, Kqiku L, Spalj S. Salivary levels of TNF-α and IL-6 in patients with oral premalignant and malignant lesions. Folia Biol. 2013;59(2):99–102.
44. Abbas AK, Lichtman AH, Pillai S. Basic immunology functions and disorders of the immune System. In: Cellular and molecular immunology. Philadelphia: Elsevier/Saunders; 2006.
45. Young MR, Levingston CA, Johnson SD. Treatment to sustain a Th17-type phenotype to prevent skewing toward Treg and to limit premalignant lesion progression to cancer. Int J Cancer. 2016;138(10):2487–98.
46. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol. 2012;12:253–68.
47. Johnson SD, Young MR. Indomethacin treatment of mice with premalignant oral lesions sustains cytokine production and slows progression to cancer. Front Immunol. 2016;7:379.
48. Parham P. The immune system. 3rd ed. New York: Garland Science; 2009.
49. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, Yokoyama WM, Ugolini S. Innate or adaptive immunity? Example of natural killer cells. Science. 2011;331:44–9.
50. Yokoyama WM, Plougastel BF. Immune functions encoded by the natural killer gene complex. Nat Rev Immunol. 2003;3:304–16.
51. Jeannin P, Jaillon S, Delneste Y. Pattern recognition receptors in the immune response against dying cells. Curr Opin Immunol. 2008;20:530–7.
52. Gardai SJ, McPhillips KA, Frasch SC, Janssen WJ, Starefeldt A, Murphy-Ullrich JE, Bratton DL, Oldenborg PA, Michalak M, Henson PM. Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on the phagocyte. Cell. 2005;123:321–34.
53. Hammes LS, Tekmal RR, Naud P, Edelweiss MI, Kirma N, Valente PT, Syrjanen KJ, Cunha-Filho JS. Macrophages, inammation and risk of cervical intraepithelial neoplasia (CIN) progression – clinicopathological correlation. Gynecol Oncol. 2007;105:157–65.
54. Kobayashi A, Weinberg V, Darragh T, Smith-McCune K. Evolving immunosuppressive microenvironment during human cervical carcinogenesis. Mucosal Immunol. 2008;1:412–20.
55. Ostrand-Rosenberg S. Immune surveillance: a balance between protumor and antitumor immunity. Curr Opin Genet Dev. 2008;18:11–8.
56. Montero AJ, Diaz-Montero CM, Kyriakopoulos CE, Bronte V, Mandruzzato S. Myeloid-derived suppressor cells in cancer patients: a clinical perspective. J Immunother. 2012;35:107–15.
57. Nelson BH. CD20(+) B cells: other tumor-infiltrating lymphocytes. J Immunol. 2010;185:4977–82.
58. June CH, Bluestone JA, Nadler LM, Thompson CB. B7 and CD28 receptor families. Immunol Today. 1994;15:321–31.
59. Keene JA, Forman J. Helper activity is required for the in vivo generation of cytotoxic T lymphocytes. J Exp Med. 1982;155:768–82.
60. Bennett SR, Carbone FR, Karamalis F, Miller JF, Heath WR. Induction of a CD8+ cytotoxic T lymphocyte response by cross-priming requires cognate CD4+ T cell help. J Exp Med. 1997;186:65–70.
61. Schoenberger SP, Toes RE, van der Voort EI, O'ringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature. 1998;393:480–3.
62. Zou W, Restifo NP. T(h)17 cells in tumour immunity and immunotherapy. Nat Rev Immunol. 2010;10:248–56.

63. Burnet M. Immunological factors in the process of carcinogenesis. Br Med Bull. 1964;20:154–8. ii general introduction 31
64. Burnet M. Cancer; a biological approach. I. Processes of control. Br Med J. 1957;1:779–86.
65. Burnet FM. Concept of immunological surveillance. Prog Exp Tumor Res. 1970;13:1–27.
66. Boon T, Cerottini JC, van den Eynde B, van der Bruggen P, Van PA. Tumor antigens recognized by T lymphocytes. Annu Rev Immunol. 1994;12:337–65.
67. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3:991–8.
68. Kim R, Emi M, Tanabe K. Cancer immunoediting from immune surveillance to immune escape. Immunology. 2007;121:1–14.
69. Pages F, Galon J, Dieu-Nosjean MC, Tartour E, Sautes-Fridman C, Fridman WH. Immune infiltration in human tumors: a prognostic factor that should not be ignored. Oncogene. 2010;29:1093–102.
70. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, Tosolini M, Camus M, Berger A, Wind P, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313:1960–4.
71. Donnem T, Hald SM, Paulsen EE, Richardsen E, Al-Saad S, Kilvaer TK, Brustugun OT, Helland A, Lund-Iversen M, Poehl M, et al. Stromal CD8(+) t-cell density-a promising supplement to TNM staging in non-small cell lung cancer. Clin Cancer Res. 2015;21:2635–43.
72. Ladanyi A, Sebestyen T, Balatoni T, Varga A, Olah J, Liszkay G. Tumor-infiltrating immune cells as potential biomarkers predicting response to treatment and survival in patients with metastatic melanoma receiving ipilimumab therapy. Eur J Cancer. 2015;51:S111–2.
73. Piersma SJ, Jordanova ES, van Poelgeest MIE, Kwappenberg KMC, van der Hulst JM, Drij'out JW, Melief CJM, Kenter GG, Fleuren GJ, O'ringa R, et al. High number of intraepithelial CD8(+) tumor-infiltrating lymphocytes is associated with the absence of lymph node metastases in patients with large early-stage cervical cancer. Cancer Res. 2007;67:354–61.
74. Galon J, Fridman WH, Pages F. Adaptive immunologic microenvironment in colorectal cancer: a novel perspective. Cancer Res. 2007;67:1883–6.
75. Galon J, Pages F, Marincola FM, Angell HK, Thurin M, Lugli A, Zlobec I, Berger A, Bifulco C, Botti G, et al. Cancer classification using the immunoscore: a worldwide task force. J Transl Med. 2012;10:205.
76. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–74.
77. zur Hausen H. Papillomavirus infections – a major cause of human cancers. Biochim Biophys Acta. 1996;1288:F55–78.
78. Brown DR, Shew ML, Qadadri B, Neptune N, Vargas M, Tu W, Juliar BE, Breen TE, Fortenberry JD. A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women. J Infect Dis. 2005;191:182–92.
79. Koutsky L. Epidemiology of genital human papillomavirus infection. Am J Med. 1997;102:3–8.
80. Chung CH, Gillison ML. Human papillomavirus in head and neck cancer: its role in pathogenesis and clinical implications. Clin Cancer Res. 2009;15:6758–62.
81. Egawa N, Egawa K, Griffin H, Doorbar J. Human papillomaviruses; epithelial tropisms, and the development of neoplasia. Viruses. 2015;7:3863–90.
82. Doorbar J, Egawa N, Griffin H, Kranjec C, Murakami I. Human papillomavirus molecular biology and disease association. Rev Med Virol. 2015;25(Suppl 1):2–23.
83. Doorbar J. Molecular biology of human papillomavirus infection and cervical cancer. Clin Sci (Lond). 2006;110:525–41.
84. Palefsky JM, Gillison ML, Strickler HD. Chapter 16: HPV vaccines in immunocompromised women and men. Vaccine. 2006;24(Suppl 3):S3-140–/146.
85. Bouwes Bavinck JN, Berkhout RJ. HPV infections and immunosuppression. Clin Dermatol. 1997;15:427–37.
86. Takeuchi O, Akira S. Recognition of viruses by innate immunity. Immunol Rev. 2007;220:214–24.

87. Karim R, Tummers B, Meyers C, Biryukov JL, Alam S, Backendorf C, Jha V, O'ringa R, van Ommen GJ, Melief CJ, et al. Human papillomavirus (HPV) upregulates the cellular deubiquitinase UCHL1 to suppress the keratinocyte's innate immune response. PLoS Pathog. 2013;9:e1003384.
88. Karim R, Meyers C, Backendorf C, Ludigs K, O'ringa R, van Ommen GJ, Melief CJ, van der Burg SH, Boer JM. Human papillomavirus deregulates the response of a cellular network comprising of chemotactic and proinflammatory genes. PLoS One. 2011;6:e17848.
89. Tummers B, Goedemans R, Pelascini LPL, Jordanova ES, van Esch EMG, Meyers C, Melief CJM, Boer JM, van der Burg SH. Interferon-related developmental regulator 1 is used by human papillomavirus to suppress NF kappa B activation. Nat Commun. 2015;6:6537.
90. Fahey LM, Raff AB, Da Silva DM, Kast WM. A major role for the minor capsid protein of human papillomavirus type 16 in immune escape. J Immunol. 2009;183:6151–6.
91. Fausch SC, Da Silva DM, Rudolf MP, Kast WM. Human papillomavirus virus-like particles do not activate Langerhans cells: a possible immune escape mechanism used by human papillomaviruses. J Immunol. 2002;169:3242–9.
92. Lehtinen M, Rantala I, Toivonen A, Luoto H, Aine R, Lauslahti K, Yla-Outinen A, Romppanen U, Paavonen J. Depletion of Langerhans cells in cervical HPV infection is associated with replication of the virus. APMIS. 1993;101:833–7.
93. Zijlmans HJ, Fleuren GJ, Baelde HJ, Eilers PH, Kenter GG, Gorter A. Role of tumor derived proinflammatory cytokines GM-CSF, TNF-alpha, and IL-12 in the migration and differentiation of antigen-presenting cells in cervical carcinoma. Cancer. 2007;109:556–65.
94. O'ringa R, de Jong A, Toes RE, van der Burg SH, Melief CJ. Interplay between human papillomaviruses and dendritic cells. Curr Top Microbiol Immunol. 2003;276:215–40.
95. Tummers B, Goedemans R, Jha V, Meyers C, Melief CJ, van der Burg SH, Boer JM. CD40-mediated amplification of local immunity by epithelial cells is impaired by HPV. J Invest Dermatol. 2014;134:2918–27.
96. Carter JJ, Koutsky LA, Wipf GC, Christensen ND, Lee SK, Kuypers J, Kiviat N, Galloway DA. Natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. J Infect Dis. 1996;174:927–36.
97. Ho GYF, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med. 1998;338:423–8.
98. Welters MJ, de JA, van den Eeden SJ, van der Hulst JM, Kwappenberg KM, Hassane S, Franken KL, Drij'out JW, Fleuren GJ, Kenter G, et al. Frequent display of human papillomavirus type 16 E6-specific memory t-Helper cells in the healthy population as witness of previous viral encounter. Cancer Res. 2003;63:636–41.
99. de Jong JA, van der Burg SH, Kwappenberg KM, van der Hulst JM, Franken KL, Geluk A, van Meijgaarden KE, Drij'out JW, Kenter G, Vermeij P, et al. Frequent detection of human papillomavirus 16 E2-specific t-helper immunity in healthy subjects. Cancer Res. 2002;62:472–9.
100. Woo YL, Sterling J, Damay I, Coleman N, Crawford R, van der Burg SH, Stanley M. Characterising the local immune responses in cervical intraepithelial neoplasia: a cross-sectional and longitudinal analysis. BJOG. 2008;115:1616–21.
101. de Jong JA, van Poelgeest MI, van der Hulst JM, Drij'out JW, Fleuren GJ, Melief CJ, Kenter G, O'ringa R, van der Burg SH. Human papillomavirus type 16-positive cervical cancer is associated with impaired CD4+ T-cell immunity against early antigens E2 and E6. Cancer Res. 2004;64:5449–55. https://doi.org/10.1158/0008-5472.CAN-04-0831.
102. Woo YL, van den Hende M, Sterling JC, Coleman N, Crawford RA, Kwappenberg KM, Stanley MA, van der Burg SH. A prospective study on the natural course of low-grade squamous intraepithelial lesions and the presence of HPV16 E2-, E6- and E7-specific t-cell responses. Int J Cancer. 2010;126:133–41.
103. van der Burg SH, Piersma SJ, de JA, van der Hulst JM, Kwappenberg KM, van den Hende M, Welters MJ, Van Rood JJ, Fleuren GJ, Melief CJ, et al. Association of cervical cancer with the presence of CD4+ regulatory T cells specific for human papillomavirus antigens. Proc Natl Acad Sci U S A. 2007;104:12087–92.

104. de Vos van Steenwijk P, Piersma SJ, Welters MJP, van der Hulst JM, Fleuren G, Hellebrekers BWJ, Kenter GG, van der Burg SH. Surgery followed by persistence of high-grade squamous intraepithelial lesions is associated with the induction of a dysfunctional HPV16-specific t-cell response. Clin Cancer Res. 2008;14:7188–95.
105. van Poelgeest MI, Nijhuis ER, Kwappenberg KM, Hamming IE, Wouter DJ, Fleuren GJ, van der Zee AG, Melief CJ, Kenter GG, Nijman HW, et al. Distinct regulation and impact of type 1 t-cell immunity against HPV16 L1, E2 and E6 antigens during HPV16-induced cervical infection and neoplasia. Int J Cancer. 2006;118:675–83.
106. Monnier-Benoit S, Mauny F, Riethmuller D, Guerrini JS, Capilna M, Felix S, Seilles E, Mougin C, Pretet JL. Immunohistochemical analysis of CD4+ and CD8+ t-cell subsets in high risk human papillomavirus-associated pre-malignant and malignant lesions of the uterine cervix. Gynecol Oncol. 2006;102:22–31.
107. Bontkes HJ, Walboomers JM, Meijer CJ, Helmerhorst TJ, Stern PL. Specific HLA class I down-regulation is an early event in cervical dysplasia associated with clinical progression. Lancet. 1998;351:187–8.
108. Keating PJ, Cromme FV, Duggan-Keen M, Snijders PJ, Walboomers JM, Hunter RD, Dyer PA, Stern PL. Frequency of down-regulation of individual HLA-A and -B alleles in cervical carcinomas in relation to TAP-1 expression. Br J Cancer. 1995;72:405–11. ii general introduction 33
109. Jordanova ES, Gorter A, Ayachi O, Prins F, Durrant LG, Kenter GG, van der Burg SH, Fleuren GJ. Human leukocyte antigen class I, MHC class I chain-related molecule A, and CD8+/regulatory t-cell ratio: which variable determines survival of cervical cancer patients? Clin Cancer Res. 2008;14:2028–35.
110. Dong DD, Yang H, Li K, Xu G, Song LH, Fan XL, Jiang XL, Yie SM. Human leukocyte antigen-G (HLA-G) expression in cervical lesions: association with cancer progression, HPV 16/18 infection, and host immune response. Reprod Sci. 2010;17:718–23.
111. Guimaraes MC, Soares CP, Donadi EA, Derchain SF, Andrade LA, Silva TG, Hassumi MK, Simoes RT, Miranda FA, Lira RC, et al. Low expression of human histocompatibility soluble leukocyte antigen-G (HLA-G5) in invasive cervical cancer with and without metastasis, associated with papilloma virus (HPV). J Histochem Cytochem. 2010;58:405–11.
112. Karim R, Jordanova ES, Piersma SJ, Kenter GG, Chen L, Boer JM, Melief CJ, van der Burg SH. Tumor-expressed B7-H1 and B7-DC in relation to PD-1+ t-cell infiltration and survival of patients with cervical carcinoma. Clin Cancer Res. 2009;15:6341–7.
113. Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. Nat Rev Immunol. 2008;8:467–77.
114. Zhu C, Anderson AC, Schubart A, Xiong H, Imitola J, Khoury SJ, Zheng XX, Strom TB, Kuchroo VK. Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat Immunol. 2005;6:1245–52.
115. Piersma SJ, Welters MJ, van der Burg SH. Tumor-specific regulatory T cells in cancer patients. Hum Immunol. 2008;69:241–9.
116. Heusinkveld M, Welters MJ, van Poelgeest MI, van der Hulst JM, Melief CJ, Fleuren GJ, Kenter GG, van der Burg SH. Detection of circulating human papillomavirus-specific T cells is associated with improved survival of patients with deeply infiltrating tumors. Int J Cancer. 2011;128:379–89.
117. de Vos van Steenwijk PJD, Ramwadhdoebe TH, Goedemans R, Doorduijn EM, van Ham JJ, Gorter A, van Hall T, Kuijjer ML, van Poelgeest MIE, van der Burg SH, et al. Tumor-infiltrating CD14-positive myeloid cells and CD8-positive t-cells prolong survival in patients with cervical carcinoma. Int J Cancer. 2013;133:2884–94.
118. Hildesheim A, Herrero R, Wacholder S, Rodriguez AC, Solomon D, Bratti MC, Schiller JT, Gonzalez P, Dubin G, Porras C, et al. Effect of human papillomavirus 16/18 L1 viruslike particle vaccine among young women with preexisting infection: a randomized trial. JAMA. 2007;298:743–53.
119. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363:711–23.

120. Dong HD, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, Roche PC, Lu J, Zhu GF, Tamada K, et al. Tumor-associated B7-H1 promotes t-cell apoptosis: a potential mechanism of immune evasion. Nat Med. 2002;8:793–800.
121. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366:2443–54.
122. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369:122–33.
123. Melief CJ, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. Nat Rev Cancer. 2008;8:351–60.
124. Mocellin S, Mandruzzato S, Bronte V, Lise M, Nitti D. Part I: vaccines for solid tumours. Lancet Oncol. 2004;5:681–9.
125. Chen YT, Panarelli NC, Piotti KC, Yantiss RK. Cancer-testis antigen expression in digestive tract carcinomas: frequent expression in esophageal squamous cell carcinoma and its precursor lesions. Cancer Immunol Res. 2014;2(5):480–6.
126. Young MR, Neville BW, Chi AC, Lathers DM, Boyd GM, Day TA. Oral premalignant lesions induce immune reactivity to both premalignant oral lesions and head and neck squamous cell carcinoma. Cancer Immunol Immunother. 2007;56:1077–86.
127. I. Young MR. Use of carcinogen-induced premalignant oral lesions in a dendritic cell-based vaccine to stimulate immune reactivity against both premalignant oral lesions and oral cancer. J Immunother. 2008;31:148–56.
128. Hanke CW, Swanson N, Bruce S, Berman B, Kulp J, Levy S. Complete clearance is sustained for at least 12 months after treatment of actinic keratoses of the face or balding scalp via daily dosing with imiquimod 3.75% or 2.50 % cream. Drugs Dermatol J. 2011;10(2):165–70.
129. Ulrich C, Johannsen A, Rowert-Huber J, Ulrich M, Sterry W, Stockfleth E. Results of a randomized placebo-controlled safety and efficacy study of topical diclofenac 3% gel in organ transplant patients with multiple keratoses. Eur J Dermatol. 2010;20(4):482–8.
130. Ghanghas P, Jain S, Rana C, Sanyal SN. Chemopreventive action of non-steroidal anti-inflammatory drugs on the inflammatory pathways in colon cancer. Biomed Pharmacother. 2016;78:239–47.
131. Toller IM, Hitzler I, Sayi A, Mueller A. 2, prevents Helicobacter-induced gastric preneoplasia and facilitates persistent infection in a mouse model. Gastroenterology. 2010;138(4). 1455–1467, 1467.e1451–1454
132. Masclee GM, Coloma PM, Spaander MC, Kuipers EJ, Sturkenboom MC. BMJ Open. 2015;5(1):e006640.
133. Thrift AP, Anderson LA, Murray LJ, Cook MB, Shaheen NJ, Rubenstein JH. Nonsteroidal anti-inflammatory drug use is not associated with reduced risk of Barrett's esophagus. Am J Gastroenterol. 2016;111(11):1528–35.
134. Zhang S, Zhang XQ, Ding XW, Yang RK, Huang SL, Kastelein F. Cyclooxygenase inhibitors use is associated with reduced risk of esophageal adenocarcinoma in patients with Barrett's esophagus: a meta-analysis. Br J Cancer. 2014;110(9):2378–88.
135. de Vos van Steenwijk PJ, van Poelgeest MI, Ramwadhdoebe TH, Lowik MJ, Berends-van der Meer DM, van der Minne CE. The long-term immune response after HPV16 peptide vaccination in women with low-grade pre-malignant disorders of the uterine cervix: a placebo-controlled phase II study. Cancer Immunol Immunother. 2014;63(2):147–60.
136. Marquez JP, Rivera R, Kang KH, Gardner MB, Torres JV. Human papillomavirus immunogen that provides protective tumor immunity and induces tumor regression. Viral Immunol. 2012;25(2):141–52.
137. De Costa AM, Justis DN, Schuyler CA, Young MR. Administration of a vaccine composed of dendritic cells pulsed with premalignant oral lesion lysate to mice bearing carcinogen-induced premalignant oral lesions stimulates a protective immune response. Int Immunopharmacol. 2012;13(3):322–30.

138. Disis ML, Gad E, Herendeen DR, Lai VP, Park KH, Cecil DL. A multiantigen vaccine targeting neu, IGFBP-2, and IGF-IR prevents tumor progression in mice with preinvasive breast disease. Cancer Prev Res. 2013;6(12):1273–82.
139. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. GLOBOCAN 2012 v1.0, cancer incidence and mortality worldwide. IARC CancerBase No. 11 [Internet]. 2013, Lyon: International Agency for Research on Cancer. Available from: http://globocan.iarc.fr. Accessed on 20 July 2018.
140. Li N, Franceschi S, Howell-Jones R, Snijders PJF, Clifford GM. Human papillomavirus type distribution in 30, 848 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication. Int J Cancer. 2010;128:927–35.
141. Bosch FX, De Sanjosé S. Chapter 1: Human papillomavirus and cervical cancer – burden and assessment of causality. J Natl Cancer Inst Monogr. 2003;(31):3–13.
142. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine. 2006;24(Suppl 1):S1–S15.
143. Gravitt PE. The known unknowns of HPV natural history. J Clin Invest. 2011;121:4593–9. https://doi.org/10.1172/JCI57149.
144. Ellerbrock TV, Chiasson MA, Bush TJ, Sun XW, Sawo D, Brudney K, Wright TC. Incidence of cervical squamous intraepithelial lesions in HIV-infected women. JAMA. 2000;283:1031–7. https://doi.org/10.1001/jama.283.8.1031.
145. Ognenovski VM, Marder W, Somers EC, Johnston CM, Farrehi JG, Selvaggi SM, McCune WJ. Increased incidence of cervical intraepithelial neoplasia in women with systemic lupus erythematosus treated with intravenous cyclophosphamide. J Rheumatol. 2004;31:1763–7.
146. Nakagawa M, Gupta SK, Coleman HN, Sellers MA, Banken JA, Greenfield WW. A favorable clinical trend is associated with CD8 T-cell immune responses to the human papillomavirus type 16 e6 antigens in women being studied for abnormal pap smear results. J Low Genit Tract Dis. 2010;14:124–9. https://doi.org/10.1097/LGT.0b013e3181c6f01e.
147. Wang SS, Schiffman M, Herrero R, Carreon J, Hildesheim A, Rodriguez AC, Bratti MC, Sherman ME, Morales J, Guillen D, Alfaro M, Clayman B, Burk RD, Viscidi RP. Determinants of human papillomavirus 16 serological conversion and persistence in a population-based cohort of 10 000 women in Costa Rica. Br J Cancer. 2004;91:1269–74. https://doi.org/10.1038/sj.bjc.6602088.
148. Carter JJ, Madeleine MM, Shera K, Schwartz SM, Cushing-Haugen KL, Wipf GC, Porter P, Daling JR, JK MD, Galloway DA. Human papillomavirus 16 and 18 L1 serology compared across anogenital cancer sites. Cancer Res. 2001;61:1934–40.
149. Stern PL, van der Burg SH, Hampson IN, Broker TR, Fiander A, Lacey CJ, Kitchener HC, Einstein MH. Therapy of human papillomavirus-related disease. Vaccine. 2012;30(Suppl 5):F71–82.
150. Kim KH, Greenfield WW, Cannon MJ, Coleman HN, Spencer HJ, Nakagawa M. CD4+ T-cell response against human papillomavirus type 16 E6 protein is associated with a favorable clinical trend. Cancer Immunol Immunother. 2012;61:63–70. https://doi.org/10.1007/s00262-011-1092-5.
151. Farhat S, Nakagawa M, Moscicki AB. Cell-mediated immune responses to human papillomavirus 16 E6 and E7 antigens as measured by interferon gamma enzyme-linked immunospot in women with cleared or persistent human papillomavirus infection. Int J Gynecol Cancer. 2009;19:508–12. https://doi.org/10.1111/IGC.0b013e3181a388c4.
152. Heusinkveld M, Welters MJ, Van Poelgeest MI, van der Hulst JM, Melief CJ, Fleuren GJ, Kenter GG, van der Burg SH. The detection of circulating human papillomavirus-specific T cells is associated with improved survival of patients with deeply infiltrating tumors. Int J Cancer. 2011;128:379–89. https://doi.org/10.1002/ijc.25361.
153. Venuti A, Paolini F, Nasir L, Corteggio A, Roperto S, Campo MS, Borzacchiello G. Papillomavirus E5: the smallest oncoprotein with many functions. Mol Cancer. 2011;10:140. https://doi.org/10.1186/1476-4598-10-140.

154. Ashrafi GH, Haghshenas MR, Marchetti B, O'Brien PM, Campo MS. The E5 protein of human papillomavirus type 16 selectively down-regulates surface HLA class. Int J Cancer. 2005;113:276–83. https://doi.org/10.1002/ijc.20558.
155. Zhang B, Li P, Wang E, Brahmi Z, Dunn KW, Blum JS, Roman A. The E5 protein of human papillomavirus type 16 perturbs MHC class II antigen maturation in human foreskin keratinocytes treated with interferon-γ. Virology. 2003;310:100–8. https://doi.org/10.1016/S0042-6822(03)00103-X.
156. Campo MS, Graham SV, Cortese MS, Ashrafi GH, Araibi EH, Dornan ES, Miners K, Nunes C, Man S. HPV-16 E5 down-regulates expression of surface HLA class I and reduces recognition by CD8 T cells. Virology. 2010;407:137–42. https://doi.org/10.1016/j.virol.2010.07.044.
157. Stanley MA, Pett MR, Coleman N. HPV: from infection to cancer. Biochem Soc Trans. 2007;35:1456–60. https://doi.org/10.1042/BST0351456.
158. O'Brien PM, Campo MS. Evasion of host immunity directed by papillomavirus encoded proteins. Virus Res. 2002;1:103–18.
159. Crosbie EJ, Einstein MH, Franceschi S, Kitchener HC. Human papillomavirus and cervical cancer. Lancet. 2013;382:889–99. https://doi.org/10.1016/S0140-6736(13)60022-7.
160. Scott ME, Ma Y, Kuzmich L, Moscicki AB. Diminished IFN-gamma and IL-10 and elevated Foxp3 mRNA expression in the cervix are associated with CIN 2 or 3. Int J Cancer. 2009;124:1379–83. https://doi.org/10.1002/ijc.24117.
161. Gooden M, Lampen M, Jordanova ES, Leffers N, Trimbos JB, van der Burg SH, Nijman H, Van Hall T. HLA-E expression by gynecological cancers restrains tumor-infiltrating $CD8^+$ T lymphocytes. Proc Natl Acad Sci U S A. 2011;108:10656–61. https://doi.org/10.1073/pnas.1100354108.
162. Piersma SJ. Immunosuppressive tumor microenvironment in cervical cancer patients. Cancer Microenviron. 2011;4:361–75. https://doi.org/10.1007/s12307-011-0066-7.
163. O'Hagan DT, Rappuoli R. Novel approaches to vaccine delivery. Pharm Res. 2004;21:1519–30.
164. Vici P, Mariani L, Sergi D, et al. Immunologic treatments for precancerous lesions and uterine cervical cancer. J Exp Clin Cancer Res. 2014;33:29.
165. Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, Chiacchierini LM, Jansen KU. Proof of principle study investigators: a controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med. 2002;347 https://doi.org/10.1056/NEJMoa020586.
166. Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, Zahaf T, Innis B, Naud P, De Carvalho NS, Roteli-Martins CM, Teixeira J, Blatter MM, Korn AP, Quint W, Dubin G, GlaxoSmithKline HPV Vaccine Study Group. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet. 2004;364:1757–65. https://doi.org/10.1016/S0140-6736(04)17398-4.
167. Lowy DR, Schiller JT. Reducing HPV-associated cancer globally. Cancer Prev Res. 2012;5:18–23. https://doi.org/10.1158/1940-6207.CAPR-11-0542.
168. Harper DM, Williams KB. Prophylactic HPV vaccines: current knowledge of impact on gynecologic premalignancies. Discov Med. 2010;10:7–17.
169. Campo MS, Roden RB. Papillomavirus prophylactic vaccines: established successes, new approaches. J Virol. 2010;84:1214–20. https://doi.org/10.1128/JVI.01927-09.
170. Vici P, Mariani L, Pizzuti L, Sergi D, Di Lauro L, Vizza E, Tomao F, Tomao S, Mancini E, Vincenzoni C, Barba M, Maugeri-Saccà M, Giovinazzo G, Venuti A. Emerging biological treatments for uterine cervical carcinoma. J Cancer. 2014;5:86–97. https://doi.org/10.7150/jca.7963.
171. Wright TC, Cox JT, Massad LS. Consensus guidelines for the management of women with cervical cytological abnormalities. JAMA. 2001;2002(287):2120–9.
172. Ma B, Maraj B, Tran NP, Knoff J, Chen A, Alvarez RD, Hung CF, Wu TC. Emerging human papillomavirus vaccines. Expert Opin Emerg Drugs. 2012;17:469–92. https://doi.org/10.1517/14728214.2012.744393.

173. Hall AH, Alexander KA. RNA interference of human papillomavirus type 18 E6 and E7 induces senescence in HeLa cells. J Virol. 2003;77:6066–9. https://doi.org/10.1128/JVI.77.10.6066-6069.2003.
174. Qi Z, Xu X, Zhang B, Li Y, Liu J, Chen S, Chen G, Huo X. Effect of simultaneous silencing of HPV-18 E6 and E7 on inducing apoptosis in HeLa cells. Biochem Cell Biol. 2010;88:697–704. https://doi.org/10.1139/O10-005.
175. Griffin H, Elston R, Jackson D, Ansell K, Coleman M, Winter G, Doorbar J. Inhibition of papillomavirus protein function in cervical cancer cells by intrabody targeting. J Mol Biol. 2006;355:360–78. https://doi.org/10.1016/j.jmb.2005.10.077.
176. Accardi L, Donà MG, Di Bonito P, Giorgi C. Intracellular anti-E7 human antibodies in single-chain format inhibit proliferation of HPV16-positive cervical carcinoma cells. Int J Cancer. 2005;116:564–70. https://doi.org/10.1002/ijc.21052.
177. Accardi L, Paolini F, Mandarino A, Percario Z, Bonito PD, Carlo VD, Affabris E, Giorgi C, Amici C, Venuti A. In vivo antitumor effect of an intracellular single-chain antibody fragment against the E7 oncoprotein of human papillomavirus 16. Int J Cancer 2014;134:2742–7.
178. Richter CE, Cocco E, Bellone S, Bellone M, Casagrande F, Todeschini P, Rüttinger D, Silasi DA, Azodi M, Schwartz PE, Rutherford TJ, Pecorelli S, Santin AD. Primary cervical carcinoma cell lines overexpress epithelial cell adhesion molecule (EpCAM) and are highly sensitive to immunotherapy with MT201, a fully human monoclonal anti-EpCAM antibody. Int J Gynecol Cancer. 2010;20:1440–7.
179. Badaracco G, Venuti A. Human papillomavirus therapeutic vaccines in head and neck tumors. Expert Rev Anticancer Ther. 2007;7:753–66. https://doi.org/10.1586/14737140.7.5.753.
180. Venuti A. Progress and challenges in the vaccine-based treatment of head and neck cancers. J Exp Clin Cancer Res. 2009;28:69. https://doi.org/10.1186/1756-9966-28-69.
181. Cheever MA, Higano CS. PROVENGE (sipuleucel-T) in prostate cancer: the first FDA 7 approved therapeutic cancer vaccine. Clin Cancer Res. 2011;17:3520–6. https://doi.org/10.1158/1078-0432.CCR-10-3126.
182. McKarney I, Sipuleucel T. Provenge: active cellular immunotherapy for advanced prostate 9 cancer. Issues Emerg Health Technol. 2007;10:1–4.
183. Ferrara A, Nonn M, Sehr P, Schreckenberger C, Pawlita M, Durst M, Schneider A, Kaufmann AM. Dendritic cell-based tumor vaccine for cervical cancer II: results of a clinical 12 pilot study in 15 individual patients. J Cancer Res Clin Oncol. 2003;129:521–30. https://doi.org/10.1007/s00432-003-0463-5.
184. Santin AD, Bellone S, Palmieri M, Zanolini A, Ravaggi A, Siegel ER, Roman JJ, Pecorelli S, Cannon MJ. Human papillomavirus type 16 and 18 E7-pulsed dendritic cell 15 vaccination of stage IB or IIA cervical cancer patients: a phase I escalating-dose trial. J Virol. 2008;82:1968–79. https://doi.org/10.1128/JVI.02343-07.
185. Trimble CL, Peng S, Kos F, Gravitt P, Viscidi R, Sugar E, Pardoll D, Wu TC. A phase I trial of a human papillomavirus DNA vaccine for HPV16+ cervical intraepithelial neoplasia 2/3. Clin Cancer Res. 2009;15:361–7. https://doi.org/10.1158/1078-0432.CCR-08-1725.
186. Klencke B, Matijevic M, Urban RG, Lathey JL, Hedley ML, Berry M, Thatcher J, Weinberg V, Wilson J, Darragh T, Jay N, Da Costa M, Palefsky JM. Encapsulated plasmid DNA treatment for human papillomavirus 16-associated anal dysplasia: a phase I study of ZYC101. Clin Cancer Res. 2002;8:1028–37.
187. Sheets EE, Urban RG, Crum CP, Hedley ML, Politch JA, Gold MA, Muderspach LI, Cole GA, Crowley-Nowick PA. Immunotherapy of human cervical high-grade cervical intraepithelial neoplasia with microparticle-delivered human papillomavirus 16 E7 plasmid DNA. Am J Obstet Gynecol. 2003;188:916–26. https://doi.org/10.1067/mob.2003.256.
188. Garcia F, Petry KU, Muderspach L, Gold MA, Braly P, Crum CP, Magill M, Silverman M, Urban RG, Hedley ML, Beach KJ. ZYC101a for treatment of high-grade cervical intraepithelial neoplasia: a randomized controlled trial. Obstet Gynecol. 2004;103:317–26. https://doi.org/10.1097/01.AOG.0000110246.93627.17.
189. Matijevic M, Hedley ML, Urban RG, Chicz RM, Lajoie C, Luby TM. Immunization with a poly (lactide co-glycolide) encapsulated plasmid DNA expressing antigenic regions of HPV

16 and 18 results in an increase in the precursor frequency of T cells that respond to epitopes from HPV 16, 18, 6 and 11. Cell Immunol. 2011;270:62–9. https://doi.org/10.1016/j.cellimm.2011.04.005.
190. Bagarazzi ML, Yan J, Morrow MP, Shen X, Parker RL, Lee JC, Giffear M, Pankhong P, Khan AS, Broderick KE, Knott C, Lin F, Boyer JD, Draghia-Akli R, White CJ, Kim JJ, Weiner DB, Sardesai NY. Immunotherapy against HPV16/ 18 generates potent TH1 and cytotoxic cellular immune responses. Sci Transl Med. 2012;4:155ra38.
191. Bilu D, Sauder DN. Imiquimod: modes of action. Br J Dermatol. 2003;149(Suppl 66):5–8.
192. Chuang CM, Monie A, Hung CF, Wu TC. Treatment with imiquimod enhances antitumor immunity induced by therapeutic HPV DNA vaccination. J Biomed Sci. 2010;17:32. https://doi.org/10.1186/1423-0127-17-32.
193. Vici P, Pizzuti L, Mariani L, Zampa G, et al. Targeting immune response with therapeutic vaccines in premalignant lesions and cervical cancer: hope or reality from clinical studies. Expert Rev Vaccines. 2016;15(10):1327–36.
194. Hariharan MJ, Driver DA, Townsend K, Brumm D, Polo JM, Belli BA, Catton DJ, Hsu D, Mittelstaedt D, McCormack JE, Karavodin L, Dubensky TW, Chang SM, Banks TA. DNA immunization against herpes simplex virus: enhanced efficacy using a Sindbis virus-based vector. J Virol. 1998;72:950–8.
195. Brandsma JL, Shylankevich M, Su Y, Roberts A, Rose JK, Zelterman D, Buonocore L. Vesicular stomatitis virus-based therapeutic vaccination targeted to the E1, E2, E6, and E7 proteins of cottontail rabbit papillomavirus. J Virol. 2007;81:5749–58. https://doi.org/10.1128/JVI.02835-06.
196. Daemen T, Riezebos-Brilman A, Bungener L, Regts J, Dontje B, Wilschut J. Eradication of established HPV16-transformed tumours after immunisation with recombinant Semliki Forest virus expressing a fusion protein of E6 and E7. Vaccine. 2003;21:1082–8. https://doi.org/10.1016/S0264-410X(02)00558-3.
197. Berglund P, Quesada-Rolander M, Putkonen P, Biberfeld G, Thorstensson R, Liljeström P. Outcome of immunization of cynomolgus monkeys with recombinant Semliki Forest virus encoding human immunodeficiency virus type 1 envelope protein and challenge with a high dose of SHIV-4 virus. AIDS Res Hum Retrovir. 1997;13:1487–95. https://doi.org/10.1089/aid.1997.13.1487.
198. Berglund P, Smerdou C, Fleeton MN, Tubulekas I, Liljeström P. Enhancing immune responses using suicidal DNA vaccines. Nat Biotechnol. 1998;16:562–5. https://doi.org/10.1038/nbt0698-562.
199. Hsu KF, Hung CF, Cheng WF, He L, Slater LA, Ling M, Wu TC. Enhancement of suicidal DNA vaccine potency by linking Mycobacterium tuberculosis heat shock protein 70 to an antigen. Gene Ther. 2001;8:376–83. https://doi.org/10.1038/sj.gt.3301408.
200. Kim TW, Hung CF, Juang J, He L, Hardwick JM, Wu TC. Enhancement of suicidal DNA vaccine potency by delaying suicidal DNA-induced cell death. Gene Ther. 2004;11:336–42. https://doi.org/10.1038/sj.gt.3302164.
201. Herd KA, Harvey T, Khromykh AA, Tindle RW. Recombinant Kunjin virus replicon vaccines induce protective T-cell immunity against human papillomavirus 16 E7-expressing tumour. Virology. 2004;319:237–48. https://doi.org/10.1016/j.virol.2003.10.032.
202. Varnavski AN, Young PR, Khromykh AA. Stable high-level expression of heterologous genes in vitro and in vivo by noncytopathic DNA-based Kunjin virus replicon vectors. J Virol. 2000;74:4394–403. https://doi.org/10.1128/JVI.74.9.4394-4403.2000.
203. Stewart TJ, Drane D, Malliaros J. ISCOMATRIX adjuvant: an adjuvant suitable for use in anticancer vaccines. Vaccine. 2004;22:3738–43. https://doi.org/10.1016/j.vaccine.2004.03.026.
204. Cui Z, Huang L. Liposome-polycation-DNA (LPD) particle as a carrier and adjuvant for proteinbased vaccines: therapeutic effect against cervical cancer. Cancer Immunol Immunother. 2005;54:1180–90. https://doi.org/10.1007/s00262-005-0685-2.
205. Kang TH, Monie A, Wu LS. Enhancement of protein vaccine potency by in vivo electroporation mediated intramuscular injection. Vaccine. 2011;29:1082–9. https://doi.org/10.1016/j.vaccine.2010.11.063.

206. Venuti A, Massa S, Mett V, Vedova LD, Paolini F, Franconi R, Yusibov V. An E7-based therapeutic vaccine protects mice against HPV16 associated cancer. Vaccine. 2009;27:3395–7. https://doi.org/10.1016/j.vaccine.2009.01.068.
207. Massa S, Franconi R, Brandi R, Muller A, Mett V, Yusibov V, Venuti A. Anti-cancer activity of plant-produced HPV16 E7 vaccine. Vaccine. 2007;25:3018–21. https://doi.org/10.1016/j.vaccine.2007.01.018.
208. Chu NR, Wu HB, Wu T, Boux LJ, Siegel MI, Mizzen LA. Immunotherapy of a human papillomavirus (HPV) type 16 E7-expressing tumour by administration of fusion protein comprising Mycobacterium bovis bacilli Calmette-Guerin (BCG) hsp65 and HPV16 E7. Clin Exp Immunol. 2000;121:216–25. https://doi.org/10.1046/j.1365-2249.2000.01293.x.
209. Liu H, Wu BH, Rowse GJ, Emtage PC. Induction of CD4-independent E7-specific CD8+ memory response by heat shock fusion protein. Clin Vaccine Immunol. 2007;14:1013–23. https://doi.org/10.1128/CVI.00029-07.
210. Liao CW, Chen CA, Lee CN. Fusion protein vaccine by domains of bacterial exotoxin linked with a tumor antigen generates potent immunologic responses and antitumor effects. Cancer Res. 2005;65:9089–98. https://doi.org/10.1158/0008-5472.CAN-05-0958.
211. Preville X, Ladant D, Timmerman B, Leclerc C. Eradication of established tumors by vaccination with recombinant Bordetella pertussis adenylate cyclase carrying the human papillomavirus 16 E7 oncoprotein. Cancer Res. 2005;65:641–9.
212. Granadillo M, Vallespi MG, Batte A, Mendoza O, Soria Y, Lugo VM, Torrens I. A novel fusion protein-based vaccine comprising a cell penetrating and immunostimulatory peptide linked to human papillomavirus (HPV) type 16 E7 antigen generates potent immunologic and anti-tumor responses in mice. Vaccine. 2011;290:920–30.
213. Zwaveling S, Ferreira Mota SC, Nouta J. Established human papillomavirus type 16-expressing tumors are effectively eradicated following vaccination with long peptides. J Immunol. 2002;169:350–8.
214. Zhang YQ, Tsai YC, Monie A, Hung CF, Wu TC. Carrageenan as an adjuvant to enhance peptide-based vaccine potency. Vaccine. 2010;28:5212–9. https://doi.org/10.1016/j.vaccine.2010.05.068.
215. Wu CY, Yang HY, Monie A. Intraperitoneal administration of poly (I:C) with polyethylenimine leads to significant antitumor immunity against murine ovarian tumors. Cancer Immunol Immunother. 2011;60:1085–96. https://doi.org/10.1007/s00262-011-1013-7.
216. Daftarian P, Mansour M, Benoit AC. Eradication of established HPV 16-expressing tumors by a single administration of a vaccine composed of a liposome-encapsulated CTL-T helper fusion peptide in a water-in-oil emulsion. Vaccine. 2006;24:5235–44. https://doi.org/10.1016/j.vaccine.2006.03.079.
217. Barrios K, Celis E. TriVax-HPV: an improved peptide-based therapeutic vaccination strategy against human papillomavirus-induced cancers. Cancer Immunol Immunother. 2012;61:1307–17. https://doi.org/10.1007/s00262-012-1259-8.
218. Goldstone SE, Palefsky JM, Winnett MT. Activity of HspE7, a novel immunotherapy, in patients with anogenital warts. Dis Colon Rectum. 2002;45:502–7. https://doi.org/10.1007/s10350-004-6229-6.
219. Derkay CS, Smith RJ, McClay J. HspE7 treatment of pediatric recurrent respiratory papillomatosis: final results of an open-label trial. Ann Otol Rhinol Laryngol. 2005;114:730–7.
220. Roman LD, Wilczynski S, Muderspach LI. A phase II study of Hsp-7 (SGN-00101) in women with high-grade cervical intraepithelial neoplasia. Gynecol Oncol. 2007;106:558–66. https://doi.org/10.1016/j.ygyno.2007.05.038.
221. Einstein MH, Kadish AS, Burk RD. Heat shock fusion protein-based immunotherapy for treatment of cervical intraepithelial neoplasia III. Gynecol Oncol. 2007;106:453–60. https://doi.org/10.1016/j.ygyno.2007.04.038.
222. Van Doorslaer K, Reimers LL, Studentsov YY, Einstein MH, Burk RD. Serological response to an HPV16 E7 based therapeutic vaccine in women with high-grade cervical dysplasia. Gynecol Oncol. 2010;116:208–12. https://doi.org/10.1016/j.ygyno.2009.05.044.

223. Daayana S, Elkord E, Winters U. Phase II trial of imiquimod and HPV therapeutic vaccination in patients with vulval intraepithelial neoplasia. Br J Cancer. 2010;102:1129–236. https://doi.org/10.1038/sj.bjc.6605611.
224. Genticel: Genticel reaches an important milestone by launching its phase II Trial in women infected with high – risk HPV before the appearance of high grade cervical lesions. 2014. Available from http://www.genticel.com/
225. Steller MA, Gurski KJ, Murakami M. Cell-mediated immunological responses in cervical and vaginal cancer patients immunized with a lipidated epitope of human papillomavirus type 16 E7. Clin Cancer Res. 1998;4:2103–9.
226. Van Driel WJ, Ressing ME, Kenter GG. Vaccination with HPV16 peptides of patients with advanced cervical carcinoma: clinical evaluation of a phase I-II trial. Eur J Cancer. 1999;35:946–52. https://doi.org/10.1016/S0959-8049(99)00048-9.
227. Welters MJ, Kenter GG, Piersma SJ. Induction of tumor-specific CD4+ andeCD8+ T-cell immunity in cervical cancerpatients by a human papillomavirustype 16 E6 and E7 long peptides vaccine. Clin Cancer Res. 2008;14:178–87. https://doi.org/10.1158/1078-0432.CCR-07-1880.
228. Kenter GG, Welters MJ, Valentijn AR. Phase I immunotherapeutic trial with long peptides spanning the E6 and E7 sequences of high-risk human papillomavirus 16 in end-stage cervical cancer patients shows low toxicity and robust immunogenicity. Clin Cancer Res. 2008;14:169–77. https://doi.org/10.1158/1078-0432.CCR-07-1881.
229. Kenter GG, Welters MJ, Valentijn AR. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. N Engl J Med. 2009;361:1838–47. https://doi.org/10.1056/NEJMoa0810097.
230. Welters MJ, Kenter GG, De Vos Van Steenwijk PJ. Success or failure of vaccination for HPV16-positive vulvar lesions correlates with kinetics and phenotype of induced T-cell responses. Proc Natl Acad Sci U S A. 2010;107:11895–9. https://doi.org/10.1073/pnas.1006500107.
231. De Vos Van Steenwijk PJ, Ramwadhdoebe TH, Lowik MJ. A placebo-controlled randomized HPV16 synthetic long-peptide vaccination study in women with high-grade cervical squamous intraepithelial lesions. Cancer Immunol Immunother. 2012;61:1485–92. https://doi.org/10.1007/s00262-012-1292-7.
232. Franconi R, Massa S, Illiano E, Mullar A, Cirilli A, Accardi L, Di Bonito P, Giorgi C, Venuti A. Exploiting the plant secretory pathway to improve the anticancer activity of a plant-derived HPV16 E7 vaccine. Int J Immunopathol Pharmacol. 2006;19:187–97.
233. Franconi R, Di Bonito P, Dibello F, Accardi L, Muller A, Cirilli A, Simeone P, Donà MG, Venuti A, Giorgi C. Plant-derived human papillomavirus 16 E7 oncoprotein induces immune response and specific tumor protection. Cancer Res. 2002;62:3654–8.
234. Muderspach L, Wilczynski S, Roman L, Bade L, Felix J, Small LA, Kast WM, Fascio G, Marty V, Weber J. A phase I trial of a human papillomavirus (HPV) peptide vaccine for women with high-grade cervical and vulvar intraepithelial neoplasia who are HPV 16 positive. Clin Cancer Res. 2000;6:3406–16.
235. Demurtas OC, Massa S, Ferrante P, Venuti A, Franconi R, Giuliano G. A chlamydomonas-derived human papillomavirus 16 E7 vaccine induces specific tumor protection. PLoS One. 2013;8:e61473. https://doi.org/10.1371/journal.pone.0061473.
236. Fukumoto H, Irahara M. Human papilloma virus (HPV) and cervical cancer. J Med Invest. 2002;49(3–4):124–33.
237. Wilting SM, Steenbergen DM. Molecular events leading to HPV-induced high grade neoplasia. Papillomavirus Res. 2016;2:85–8.

Utility of Sentinel Node Biopsy in Cervical Cancer

8

Alejandra Mateos, Silvia Marín, and Ignacio Zapardiel

Historically, lymph node involvement in solid tumors has been deeply investigated, as it is one of the most important predictors of disease-free and overall survival in early stages. Since the nineteenth century, it has been known that in the presence of infections or tumors, bacteria and malignant cells can disseminate along the lymphatic system and reach the lymph nodes [1]. Based on this idea, complete pelvic lymphadenectomy was performed with the purpose of staging and treating some tumors. The main issue regarding this surgery is its association with important complications, so a different surgical technique which could avoid such morbidity to patients was required.

History

The term "sentinel node" was first used by Braithwaite in 1923, and it was defined as the first node that receives the lymphatic drainage of the tumor [2]. The sentinel node has been studied in other tumors before its use in cervical cancer. The first case was penile cancer, with Cabañas, in the 1970s; using lymphogammagraphy, there was the possibility to identify the lymphatic drainage, and it was observed that the majority of patients with metastases only had the sentinel node affected. Moreover, following this event it was suggested that there was no need for inguinofemoral lymphadenectomy if the sentinel node was negative [3].

The next tumor in which the sentinel node was studied was the melanoma, as it disseminates by the lymphatic system, and its most important prognostic factor is the node involvement. Nevertheless, only 15–20% of the patients had metastases at the time of diagnosis, so too many patients would have been operated without benefits.

A. Mateos · S. Marín
Gynecologic Oncology Unit, La Paz University Hospital, Madrid, Spain

I. Zapardiel (✉)
Gynecologic Oncology Unit, La Paz University Hospital-IdiPAZ, Madrid, Spain

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_8

Morton, in 1992, injected a colorant to identify the first node of drainage, with a detection rate of 81% and false-negative rate of 1% – he concluded that sentinel node biopsy was an accurate and reliable technique [4]. Some years later, a multicentric study demonstrated this conclusion by using a combined technique of technetium-99 (Tc-99) and blue dye, and nowadays, sentinel node biopsy is the preferred technique for staging and treatment of melanoma [5].

Sentinel node biopsy was applied in breast cancer in 1993, by Krag, who used and injected Tc-99 4 h before the surgery. More studies were developed for many years, and in 1998 the biopsy of sentinel node was confirmed as the ideal technique for staging and treatment of breast cancer. In 2006, Veronesi concluded with a randomized study of 516 patients that the technique is oncologically safe, and axillary lymphadenectomy is not justified in all patients [6–8].

Regarding gynecological pelvic tumors, vulvar cancer used to be treated with resection of the tumor and unilateral or bilateral inguinal lymphadenectomy, resulting in numerous complications. The sentinel node was therefore also developed in vulvar tumors. Levenback published a study in which the conclusion was that biopsy of sentinel node is a reliable technique and that scientific evidence recommends it for vulvar cancer with specific characteristics (located, less than 4 cm, no suspicious nodes) [9, 10].

Multiple studies are also now investigating the utility of sentinel node biopsy in endometrial cancer, with hopeful results.

In this chapter, we will discuss the technique, analysis, and scientific evidence on the advantages of sentinel node biopsy in cervical cancer. This procedure is relatively new in oncology, compared with other tumors, but it has developed fastly, and the results are very promising. The 2015 edition of National Comprehensive Cancer Network includes node mapping in early-stage cervical cancer, if the clinical center has the adequate equipment and experienced surgeons to carry out the technique [11].

Staging of Cervical Cancer

Staging is useful to stablish prognosis and subsequent treatment for tumors. In cervical cancer, FIGO classification is used at the time of diagnosis, but it does not include node involvement. By this classification, cervical cancer is divided into two groups: early stage (IA1, IA2, IB1, IIA1) and advanced stage (IIA2, IIB, III, IV) [12].

The most important predictor factors in cervical cancer are the disease stage and the lymph node involvement at the time of diagnosis. In early-stage disease, the incidence of lymph nodal metastases is 15–20%, with an overall survival in 5 years of 45%, instead of 90% if there is no lymphatic dissemination. Furthermore, if there is dissemination disease, patients will receive adjuvant treatment. This treatment would result in associated complications, so it would be useful to know the node involvement before surgery, to avoid as much as possible the morbidity of combined surgery and radiotherapy +/− chemotherapy [13, 14].

Image techniques are not very useful to determine node involvement. Magnetic resonance and computed tomography only detect changes in size and shape of

nodes and do not differentiate between metastases and inflammatory characteristics. Positron emission tomography imaging (PET-TAC) has more sensitivity and specificity, but it is also limited for tumoral focus less than 7 millimeters [15].

The gold standard technique for nodal staging has been, for many years, pelvic lymphadenectomy, with sensitivity and specificity of 100% [16]. It consists on removing fatty tissue around the pelvic vessels, which limits are, laterally, external iliac vessels, psoas muscle, and genitofemoral nerve; medially, ureter and umbilical or superior vesical artery; front, posterior surface of the external iliac vein; posterior, obturator artery and nerve, obturator muscle, and obturator foramen; caudal, ischiopubic branch; and cranial, iliac vessel bifurcation. This surgical technique is not exempt of complications, which can include vessel and nerve injury, ureter damage, those related to the laparoscopy, infection, and the most frequent long-term complications: lymphocele and lymphedema [17]. It was therefore necessary to develop a new procedure to avoid complications and morbidity. Histologic analysis of the first node of drainage gives information about lymphatic dissemination; if the sentinel node has no tumoral cells, the rest of the system is supposed to be free of disease. At this point, sentinel node biopsy arose, with the intention to correctly stage tumors without performing lymphadenectomy. Moreover, we can make an intraoperative analysis and detect low-volume metastases and objective lymphatic drainage in atypical regions.

The first multicentric study was published in 2008. It included 507 women with all stages of disease: the detection rate was of 94%, the sensitivity of 90.9%, and the negative predictive value of 99.1%, and in tumors of less than 2 cm, it was higher than in those of more than 2 cm. So, the conclusion was that bilateral sentinel node biopsy could replace pelvic lymphadenectomy in the future, but more studies were necessary for oncology security [18].

It is important to know the node involvement for fertility-sparing surgery in early-stage disease. Many patients who are candidates for this type of surgery do not have lymph node metastases, so biopsy of sentinel node turned up to avoid such morbidity in women for whom pelvic lymphadenectomy was not necessary. Radical trachelectomy is oncologically safe in patients with tumors of less than 2 cm, without lymphatic dissemination. When it is not possible to perform sentinel node biopsy, some surgeons perform pelvic lymphadenectomy and, secondly, the surgery [19].

In 2011, Du reported a study with 68 patients less than 41 years old, with early-stage disease and therefore candidates for fertility-sparing surgery. He concluded that sentinel node biopsy is a minimally invasive and reliable technique for staging patients, with the advantage of selecting those women who can obtain benefit from radical trachelectomy [20].

Detection and Analysis of Sentinel Node

To identify the sentinel node, it is necessary to use a substance that can migrate through the lymphatic system and reach the first node of drainage. Originally, vital dyes and radiotracers were the two substances used, but nowadays new techniques with fluorescents dyes have been developed.

In the beginning, blue dye was the first used. After being injected, it can migrate through the lymphatic vessels to the first drainage node and allow surgeons to identify the system because of the color change. To perform this technique, it is very important to have a good knowledge of the anatomy and, of course, to have experience in detailed dissection. The first specialist who published a series of cases was Dargent in 2000. He performed sentinel node biopsy in 35 patients with cervical cancer, by laparoscopy, injecting blue dye. The detection rate was of 89%, with sensitivity of 100%, without false-negative cases, so he concluded that it could be a hopeful technique [21].

Some organs have complex and bilateral drainages, like the cervix and uterus, so the detection rate of sentinel nodes with dyes can be low. To solve this problem, radiotracers started to be used. We inject radiocolloids, proteins marked with radiotracers that can migrate through the lymphatic system and reach the sentinel node and which are detected during the surgery with a gamma probe by the radioactivity they spread. The most used radiocolloid is technetium-99, due to its half-life (6 h), which means less radiation for the patient and surgeon. Many substances are marked with Tc-99, and, depending on their size, different results can be obtained. For example, small particles migrate very fast, but they disappear from the node early, allowing to detect more nodes (perhaps secondary nodes) that can result in false negatives. On the other hand, big particles migrate slower, meaning that more time is required for the surgery, but they remain more time in the node [22]. During the surgery, the sentinel node is identified with a gamma probe like the node which radioactivity is five times higher than the rest of the tissue.

In 2000, Verheijen used a combined technique of technetium-99 and blue dye in 11 patients, making a lymphogammagraphy before the surgery. He detected Tc-99-positive nodes in all women, but blue dye positives only in four, concluding that radiotracers are more sensitive [23].

The first study which compared different techniques of detection-combined technique and blue dye only was published by Plante, with higher detection rate of combined Tc-99 and blue dye, with statistically significant difference [24]. Later, more studies were developed comparing the different techniques and reached the same conclusion. Even more, some authors observed a higher detection rate with combined Tc-99 and blue dye and in tumors of less than 2 cm [25].

There are some technical aspects on mapping detection that should be considered for better sentinel node mapping. First, an important point is the time between the injection and the detection in the surgery, since it is necessary to allow for enough time for the particle to migrate. Some studies present long protocols (injection 18–24 h before surgery) and other short protocols (3–6 h). In a meta-analysis carried out by Kadkhodayan in 2014, he concluded that for blue dyes, the best time for detecting blue nodes is 30 min after injection and that, after 50 min, the detection rate decreased considerably. More time is needed for radiotracers, as protocols of up to 48 h have been described, with a slightly higher detection rate in protocols of 18–24 h. Secondly, the injection volume is also important since in the same meta-analysis, there was a conclusion that volumes lower than 1–2 mL make the detection technique more likely to fail [26].

Radiotracers are useful to have images prior to the surgery, to identify the anatomic region where the tracer has migrated and the number and location of sentinel nodes. Lymphogammagraphy was the first image technique used for this purpose. In 2010, an article objectified a low agreement between the locations of the sentinel node by lymphogammagraphy and by laparoscopy [27]. Later, Bats published a study in 2015 based on the results of the multicentric study SENTICOL that concluded that the agreement between lymphogammagraphy and laparoscopy is very low, with a kappa index of 0.23 [28]. Recently, SPECT-TAC has displaced the lymphogammagraphy, as it offers anatomic and functional images, that means less time and complications. Some authors believe that imaging techniques before surgery involve less surgical time and less complications [29]. A study carried out by Hoogendam in 2013 shows that the bilateral intraoperative sentinel node retrieval times for SPECT-CT were significantly lower than with lymphogammagraphy [30]. However, these techniques are expensive and do not have clear advantages in detecting sentinel nodes, but they can be useful for those surgeons who do not have too much experience.

Fluorescent dyes have been incorporated for the detection of sentinel nodes. Indocyanine green (ICG) is the only approved one for the FDA. It is injected near the tumor and shows fluorescence by applying light with 700–900 nanometers of wave length (near-infrared), even if the node is not on the surface of the tissue, as the fluorescence can penetrate some millimeters. It provides real-time imaging during surgery (Fig. 8.1).

Several studies have compared the overall and bilateral detection rates and false-negative rates of ICG vs radiotracer with or without blue dye, concluding that the sentinel lymph node mapping with indocyanine green has significantly higher detection rate and bilateral mapping compared to radiocolloid and blue dye technique [31, 32].

As with blue dyes and radiotracers, it is necessary to establish the technical aspects that allow for better detection rates with indocyanine green. For ICG, the best time for injection for good visualization of sentinel lymph nodes is 5–60 min before the procedure [33]. As for the injection volume, a dose-defining study in 2012 concludes that 1 ml cervical injection of ICG could identify sentinel lymph node in 88% of patients (95% CI, 64–99%) [34]. Finally, the combination of indocyanine green with albumin has been studied, concluding that there is no observed advantage of ICG-albumin over ICG alone for the sentinel lymph node procedure in early-stage cervical cancer [35].

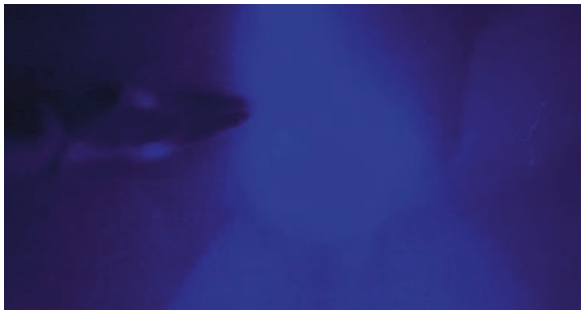

Fig. 8.1 Sentinel node identification with ICG technique. (Courtesy of Hospital Universiario La Paz)

The technique is not exempt of adverse events. There are some events described for dye colorants, the most serious being anaphylactic shock, in 1–2% of patients. Other situations include green urine and cutaneous rash. No adverse effects have been described with Tc-99 to date [24, 36].

Sentinel node biopsy can be performed by laparotomy, laparoscopy, or robotics. Laparoscopy has some advantages such as less bleeding, less postoperative pain and better surgical field, better visualization of anatomic regions, and detailed dissection. Laparoscopy and robotics have better detection rates and more sensitivity, but it is important to emphasize on the experience of the surgeon [26] (Fig. 8.2).

A meta-analysis of 67 series was published in 2015, including articles with combined technique, only dye colorants or only Tc-99, with a global detection rate of 89.2% [37, 38]. Combined technique of sentinel node detection has demonstrated better results in bilateral detection. In 2007, Hauspy published a review in which he observed only 12 false negative, and only 1 of them through bilateral detection, the others being unilateral. He concluded that false-negative results do not include unilateral detection or contralateral metastases, so it is necessary to proceed with a bilateral detection to make the sentinel node biopsy a reliable technique [39]. In 2011, a multicentric study in France published a statistical significance between combined technique and bilateral detection, patient's age (more detection in younger), and lymphovascular invasion (less detection with LVI) [40]. Consequently, the majority of authors agree that bilateral detection is necessary.

Plante affirmed in 2003 that bilateral detection and rates of detection were better with experienced surgeons, and multiple studies have concluded that the learning curve is very important [24, 39, 41]. In cervical cancer, a big number of patients are supposedly needed to complete the learning, as the cervix has a complex drainage. However, the number can be smaller if the surgeon has some experience in sentinel nodes of other tumors, like breast or vulvar cancer. There is no stablished number of patients required to complete the learning curve for cervical cancer, but there is an estimation of around 10. We cannot forget that the learning curve is also significant for oncologists, pathologists, and nuclear medicine specialists [42, 43].

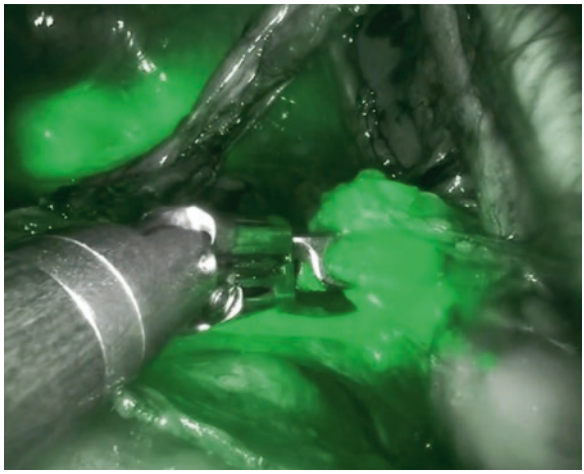

Fig. 8.2 Sentinel node by robotic approach

Similar to the terminology used to classify sentinel nodes in the pelvis, these are named by the nearest vessel: common iliac, external iliac, internal iliac, obturator, presacral, para-aortic, and parametrial. The majority of excised sentinel nodes are from internal and external iliac and obturator chains (typical drainage). Nevertheless, other nodes are described like atypical drainage, like common iliac, presacral, and para-aortic, in 20% of the cases ("skip metastases" in para-aortic nodes are very unusual). In 2003, Buist published a paper with 10% of cases with nodes in atypical locations, using combined technique of Tc-99 and blue dye [44].

Gortzak-Uzan compared sentinel node techniques with lymphadenectomy in a case-control study. He observed a higher detection of metastases with sentinel node than with pelvic lymphadenectomy, this being justified as the sentinel node technique can detect low-volume metastases by ultrastaging analysis and allow for the detection of nodes in atypical locations [45].

Nodes located in these atypical regions involve higher risk of recurrence, because when not detected by SNB, the patient is underdiagnosed. This could be the reason why 12–15% of early-stage cervical cancers without pelvic metastases have locoregional relapses [46].

Parametrial nodes are difficult to detect, even if we use dye colorants or Tc-99. Perhaps, it is easier to detect the sentinel node with dye colorant, as radiotracer is injected near the tumor, and the radioactivity is too high to identify the node. The prognostic importance of parametrial sentinel nodes is unknown, and even some authors do not believe in them. In case they exist, they are excised in the radical hysterectomy. They could be relevant if it is possible to perform a conization or a simple hysterectomy in tumors of less than 2 cm, as nowadays radical surgery is debatable [47–49].

Sentinel nodes are studied in a different way than other nodes, because the histologic analysis performed during the surgery can change the therapeutic attitude. Later, the ultrastaging study allows the pathologist to detect tumor deposits of small volume that can modify the prognosis of the patient. The intraoperative analysis allows to modify the treatment. If the sentinel node is positive, surgical treatment will be left, and para-aortic lymphadenectomy will be performed, to know radiation fields for posterior radiotherapy. If it is negative, surgical treatment and pelvic lymphadenectomy can follow [17].

The intraoperative analysis consists in having frozen cuts of the tissue and dying them with hematoxylin and eosin. The main issue with this technique, however, is that it takes time and that it has low sensitivity for the detection of small metastases. We can conclude that it is necessary to develop new ways to improve this analysis, especially for micrometastases [50].

The ultrastaging analysis is performed after the surgery, and it is very useful to detect micrometastases and isolated tumoral cells. There are three levels described for ultrastaging: histologic (H&E), immunohistochemistry (cytokeratins), and molecular (mRNA amplification, OSNA). In a meta-analysis made by Tax in 2015, a sensitivity of 94% is shown with ultrastaging, with 19 false negatives out of 1275 patients. The risk of undertreatment was subsequently estimated in 1.5%. However, with additional criteria like bilateral sentinel lymph node detection (early stage), no suspicious lymph nodes during surgery, and tumor size less than 4 cm, the risk of occult metastases and, thus, undertreatment decreases to 0.08% [51].

We followed the criteria of the American Joint Committee on Cancer (AJCC) for breast cancer to define low-volume disease in cervix cancer, as we do not have specific criteria for this tumor. Macrometastases are defined as tumoral deposits of more than 2 mm, micrometastases between 2 and 0.2 mm, and isolated tumoral cells smaller than 0.2 mm [17]. The prevalence of micrometastases in sentinel nodes is 4–15% [52].

In 2003, Barranger published the first study with immunohistochemical analysis for lymph nodes, which detected low-volume metastases in more patients than the usual technique [53]. Horn, in 2008, published a series with almost 900 patients with a follow-up of 84 months, concluding a higher risk of recurrence and death of disease in those women with micrometastasis, suggesting that radical surgery must be displaced by radio- and chemotherapy [54]. In 2012, Cibula published a multicentric, retrospective cohort study with 645 women with early-stage cervical cancer. His objective was to determine the prognostic significance of micrometastases and isolated tumor cells in sentinel nodes, observing decreased survival with micrometastases, similar to what applies to macrometastases. Thus, sentinel node biopsy can detect those patients with micrometastases, who could not be diagnosed with usual histologic analysis [55]. Even more, based on this study, patients with micrometastases must be treated with radiotherapy and chemotherapy, because they are no candidates for surgical treatment.

The technique usually uses pancytokeratin antibodies E1/E3. Epidermoid cervical cancer is derived from the epithelium, so there are cytokeratins in the tissue, but lymphatic nodes do not express these proteins if there is no disseminated disease. These techniques are expensive, and they are not profitable if they are used in all nodes, but if this analysis is applied in a limited number of nodes, we obtain prognostic information [56].

If no tumoral deposits are seen in histologic and ultrastaging analysis, the sentinel node is considered negative, but in case the pathologist objectifies any of these deposits, it is necessary to specify the volume.

The Newest Findings About Sentinel Nodes in Cervical Cancer

Despite all the studies carried out on the sentinel lymph node biopsy in cervical cancer, this technique remains novel and suggests further studies exploring new fields.

As already mentioned, the high detection rate and low number of false negatives for sentinel node biopsy have been demonstrated in early stages of cervical cancer (FIGO stage <IB1). The detection rate from radiotracer and blue dye techniques is lower in patients with locally advanced cervical cancer, decreasing to 65.9%, because of lymph node involvement and lymphovascular invasion, which hampers lymphatic flow [26]. Due to its low accuracy, sentinel node mapping is not a validated technique in advanced cervical cancer.

However, with the appearance of ICG, the detection rate of sentinel lymph node with ICG compared to traditional techniques in advanced stages has begun to be studied. Di Martino published a multicenter study that includes 95 patients, mapped

with Tc-99 radiotracer (with or without blue dye) or mapped with ICG. They conclude that in FIGO stages > IB1, the fluorescent dye technique demonstrated a higher detection rate than radiotracer with or without blue dye (100% vs 91.5%), with greater bilateral mapping (91.7% vs 66.0%) [57]. More studies would be required, but this could extend the sentinel lymph node biopsy.

There are other branches of study, although with little data at the moment. A Chinese group studied detection rate and accuracy using carbon nanoparticles instead of ICG, radiotracer, or blue dye, with good results, but in a small sample [58]. A Spanish group evaluated the role of indocyanine green combined with Tc-99, with a bilateral detection rate of 100% [59].

Conclusion

Multiple studies have been published, including 1000 of patients for whom biopsy of sentinel node has been performed. This technique has been demonstrated as a feasible procedure, with high sensitivity and predictive negative value, using combined technique of Tc-99 and blue dye, performing bilateral detection and done by experienced surgeons. Also, it allows to detect atypical location nodes and low-volume metastases, which is important for treatment and prognosis. Nevertheless, it is fundamental to develop new techniques for intraoperative study.

Is it the end of lymphadenectomy in early-stage disease? There is not enough scientific evidence to decide to leave pelvic lymphadenectomy, but some groups are now performing only sentinel node biopsy, instead of lymphadenectomy, in patients with tumors smaller than 2 cm, without lymphovascular dissemination and bilateral sentinel node negatives [60].

The SENTICOL 2 is being developed, with the objective of evaluating sentinel node biopsy only versus sentinel node plus lymphadenectomy. Surely, new and interesting results will be obtained from it.

References

1. Virchow R. Lecture IX: Pyanemia and leucocytosipathologicals. Cellular pathology as based upon physiology and history. New Cork: Dover Publ Inc; 1971. p. 221–9.
2. Braithwaite LR. The flow of lymph from ileocecal angle and its possible bearing on the cause of duodenal and gastric ulcer. Br J Surg. 1923;11:117–26.
3. Cabañas RM. Anatomy and biopsy of sentinel lymph nodes. Urol Clin North Am. 1992;19(2):267–76.
4. Morton DL, Wen DR, Wong JH, Economou JS, Cagle LA, Storm FK, Foshag LJCA. Technical details of intraoperative lymphatic mapping for early stage melanoma. Arch Surg. 1992;127(4):392–9.
5. Morton DL, Thompson JF, Essner R, Elashoff R, Stern SL, Nieweg OE, Multicenter Selective Lymphadenectomy Trial Group, et al. Validation of the accuracy of intraoperative lymphatic mapping and sentinel lymphadenectomy for early-stage melanoma: a multicenter trial. Ann Surg. 1999;230(4):453–63; discussion 463–465
6. Krag DN, Weaver DL, Alex JC, Fairbank JT. Surgical resection and radiolocalization of the sentinel lymph node in breast cancer using a gamma probe. Surg Oncol. 1993;2(6):335–9; discussion 340

7. Mansel RE, Fallowfield L, Kissin M, Goyal A, Newcombe RG, Dixon JM, et al. Randomized multicenter trial of sentinel node biopsy versus standard axillary treatment in operable breast cancer: the ALMANAC Trial. J Natl Cancer Inst. 2006;98(9):599–609.
8. Veronesi U, Paganelli G, Viale G, Luini A, Zurrida S, Galimberti V, et al. Sentinellymph-node biopsy as a staging procedure in breast cancer: update of a randomised controlled study. Lancet Oncol. 2006;7(12):983–90.
9. Levenback C, Burke TW, Morris M, Malpica A, Lucas KR, Gershenson DM. Potential applications of intraoperative lymphatic mapping in vulvar cancer. Gynecol Oncol. 1995;59(2):216–20.
10. Covens A, Vella ET, Kennedy EB, Reade CJ, Jimenez W, Le T. Sentinel lymph node biopsy in vulvar cancer: Systematic review, meta-analysis and guideline recommendations. Gynecol Oncol. 2015;137(2):351–61.
11. NCCN Clinical Practice Guidelines in Oncology. Cervical cancer, Version 3. 2019.
12. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynecol Obstet. 2009;105(2):103–4.
13. Eiriksson LR, Covens a. Sentinel lymph node mapping in cervical cancer: the future? BJOG. 2012;119(2):129–33.
14. Landoni F, Maneo A, Colombo A, Placa F, Milani R, Perego P, et al. Randomised study of radical surgery versus radiotherapy for stage Ib-IIa cervical cancer. Lancet. 1997;350(9077):535–40.
15. Chung HH, Park N-H, Kim JW, Song Y-S, Chung J-K, Kang S-B. Role of integrated PET-CT in pelvic lymph node staging of cervical cancer before radical hysterectomy. Gynecol Obstet Investig. 2009;67(1):61–6.
16. Dekindt C, Stoeckle E, Thomas L, Floquet A, Kind M, Brouste V, et al. Laparoscopic interiliacal lymphadenectomy in cancer of the uterine cervix: still the gold standard? A propos lymph node recurrences in 190 treated patients. J Gynecol Obstet Biol Reprod (Paris). 2005;34(5):473–80.
17. Fresno S, Vidal H. Cirugía Ginecológica para residentes 2016. Ed SEGO (Spain). p. 155–174.
18. Altgassen C, Hertel H, Brandstädt A, Köhler C, Dürst M, Schneider A. Multicenter validation study of the sentinel lymph node concept in cervical cancer: AGO Study Group. J Clin Oncol. 2008;26(18):2943–51.
19. Pareja R, Rendón GJ, Sanz-Lomana CM, Monzón O, Ramirez PT. Surgical, oncological, and obstetrical outcomes after abdominal radical trachelectomy – a systematic literature review. Gynecol Oncol. 2013;131(1):77–82.
20. Du X-L, Sheng X-G, Jiang T, Li Q-S, Yu H, Pan C-X, et al. Sentinel lymph node biopsy as guidance for radical trachelectomy in young patients with early stage cervical cancer. BMC Cancer. 2011;11:157.
21. Dargent D, Martin X, Mathevet P. Laparoscopic assessment of the sentinel lymph node in early stage cervical cancer. Gynecol Oncol. 2000;79(3):411–5.
22. De Cicco C, Cremonesi M, Chinol M, Bartolomei M, Pizzamiglio M, Leonardi L, et al. Optimization of axillary lymphoscintigraphy to detect the sentinel node in breast cancer. Tumori. 1997;83(2):539–41.
23. Verheijen RH, Pijpers R, Van DPJ, Burger CW, Buist MR, Kenemans P. Sentinel node detection in cervical cancer. Obstet Gynecol. 2000;96(1):135–8.
24. Plante M, Renaud M-C, Têtu B, Harel F, Roy M. Laparoscopic sentinel node mapping in early-stage cervical cancer. Gynecol Oncol. 2003;91(3):494–503.
25. Rob L, Strnad P, Robova H, Charvat M, Pluta M, Schlegerova D, et al. Study of lymphatic mapping and sentinel node identification in early stage cervical cancer. Gynecol Oncol. 2005;98(2):281–8.
26. Kadkhodayan S, Hasanzadeh M, Treglia G, et al. Sentinel node biopsy for lymph nodal staging of uterine cervix cancer: A systematic review and meta-analysis of the pertinent literature. Eur J Surg Oncol. 2015;41(1):1–20.
27. Fotiou S, Zarganis P, Vorgias G, Trivizaki E, Velentzas K, Akrivos T, et al. Clinical value of preoperative lymphoscintigraphy in patients with early cervical cancer considered for intraoperative lymphatic mapping. Anticancer Res. 2010;30(1):183–8.

28. Bats A-S, Frati A, Mathevet P, Orliaguet I, Querleu D, Zerdoud S, et al. Contribution of lymphoscintigraphy to intraoperative sentinel lymph node detection in early cervical cancer: Analysis of the prospective multicenter SENTICOL cohort. Gynecol Oncol. 2015;137(2):264–9.
29. Klapdor R, Mücke J, Schneider M, Länger F, Gratz K-F, Hillemanns P, et al. Value and advantages of preoperative sentinel lymph node imaging with SPECT/CT in cervical cancer. Int J Gynecol Cancer. 2014;24(2):295–302.
30. Hoogendam JP, Hobbelink MG, Veldhuis WB, et al. Preoperative sentinel node mapping with (99m)Tc-nanocolloid SPECT-CT significantly reduces the intraoperative sentinel node retrieval time in robot assisted laparoscopic cervical cancer surgery. Gynecol Oncol. 2013;129:389–94.
31. Ruscito I, Gasparri ML, Braicu EI, et al. Sentinel node mapping in cervical and endometrial cancer: indocyanine green versus other conventional dyes-a meta-analysis. Ann Surg Oncol. 2016;23:3749–56.
32. Buda A, Papadia A, Zapardiel I, et al. From conventional radiotracer tc-99m with blue dye to indocyanine green fluorescence: a comparison of methods towards optimization of sentinel lymph node mapping in early stage cervical cancer for a laparoscopic approach. Ann Surg Oncol. 2016;23(9):2959–65.
33. Choi HJ, Kim TJ, Lee YY, et al. Time-lapse imaging of sentinel lymph node using indocyanine green with near-infrared fluorescence imaging in early endometrial cancer. J Gynecol Oncol. 2016;27:e27.
34. Rossi EC, Ivanova A, Boggess JF. Robotically assisted fluorescence-guided lymph node mapping with ICG for gynecologic malignancies: a feasibility study. Gynecol Oncol. 2012;124:78–82.
35. Schaafsma BE, van der Vorst JR, Gaarenstroom KN, et al. Randomized comparison of near-infrared fluorescence lymphatic tracers for sentinel lymph node mapping of cervical cancer. Gynecol Oncol. 2012;127:126–30.
36. Leong SP, Donegan E, Heffernon W, Dean S, Katz JA. Adverse reactions to isosulfan blue during selective sentinel lymph node dissection in melanoma. Ann Surg Oncol. 2000;7(5):361–6.
37. Wu Y, Li Z, Wu H, Yu J. Sentinel lymph node biopsy in cervical cancer: A metaanalysis. Mol Clin Oncol. 2013;1(6):1025–30.
38. Levenback C, Coleman RL, Burke TW, Lin WM, Erdman W, Deavers M, et al. Lymphatic mapping and sentinel node identification in patients with cervix cancer undergoing radical hysterectomy and pelvic lymphadenectomy. J Clin Oncol. 2002;20(3):688–93.
39. Hauspy J, Beiner M, Harley I, Ehrlich L, Rasty G, Covens A. Sentinel lymph nodes in early stage cervical cancer. Gynecol Oncol. 2007;105(2):285–90.
40. Lécuru F, Mathevet P, Querleu D, Leblanc E, Morice P, Daraï E, et al. Bilateral negative sentinel nodes accurately predict absence of lymph node metastasis in early cervical cancer: Results of the SENTICOL study. J Clin Oncol. 2011;29(13):1686–91.
41. Roy M, Bouchard-Fortier G, Popa I, Grégoire J, Renaud M-C, Têtu B, et al. Value of sentinel node mapping in cancer of the cervix. Gynecol Oncol. 2011;122(2):269–74.
42. Frumovitz M, Ramirez PT, Levenback CF. Lymphatic mapping and sentinel lymph node detection in women with cervical cancer. Gynecol Oncol. 2008;110(3 Suppl 2):S17–20.
43. Gien LT, Covens A, Oncology G, Health S, Centre S. Quality control in sentinel lymph node biopsy in cervical cancer. J Clin Oncol. 2008;26(18):2930–1.
44. Buist MR, Pijpers RJ, van Lingen A, van Diest PJ, Dijkstra J, Kenemans P, et al. Laparoscopic detection of sentinel lymph nodes followed by lymph node dissection in patients with early stage cervical cancer. Gynecol Oncol. 2003;90(2):290–6.
45. Gortzak-Uzan L, Jimenez W, Nofech-Mozes S, Ismiil N, Khalifa MA, Dubé V, et al. Sentinel lymph node biopsy vs. pelvic lymphadenectomy in early stage cervical cancer: is it time to change the gold standard? Gynecol Oncol. 2010;116(1):28–32.
46. Ouldamer L, Marret H, Acker O, Barillot I, Body G. Unusual localizations of sentinel lymph nodes in early stage cervical cancer: a review. Surg Oncol. 2012;21(3):153–7.

47. Winter R, Haas J, Reich O, Koemetter R, Tamussino K, Lahousen M, et al. Parametrial spread of cervical cancer in patients with negative pelvic lymph nodes. Gynecol Oncol. 2002;84(2):252–7.
48. Covens A, Rosen B, Murphy J, Laframboise S, DePetrillo AD, Lickrish G, et al. How important is removal of the parametrium at surgery for carcinoma of the cervix? Gynecol Oncol. 2002;84(1):145–9.
49. Ramirez PT, Pareja R, Rendón GJ, Millan C, Frumovitz M, Schmeler KM. Management of low-risk early-stage cervical cancer: Should conization, simple trachelectomy, or simple hysterectomy replace radical surgery as the new standard of care? Gynecol Oncol. 2014;132(1):254–9.
50. Martínez A, Mery E, Filleron T, Boileau L, Ferron G, Querleu D. Accuracy of intraoperative pathological examination of SLN in cervical cancer. Gynecol Oncol. 2013;130(3):525–9.
51. Tax C, Rovers MM, Graaf C, et al. The sentinel node procedure in early stage cervical cancer, taking the next step; a diagnostic review. Gynec Oncol. 2015;139:559–67.
52. Slama J, Dundr P, Dusek L, Cibula D. High false negative rate of frozen section examination of sentinel lymph nodes in patients with cervical cancer. Gynecol Oncol. 2013;129(2):384–8.
53. Barranger E, Grahek D, Cortez A, Talbot JN, Uzan S, Darai E. Laparoscopic sentinel lymph node procedure using a combination of patent blue and radioisotope in women with cervical carcinoma. Cancer. 2003;97(12):3003–9.
54. Horn LC, Hentschel B, Fischer U, Peter D, Bilek K. Detection of micrometastases in pelvic lymph nodes in patients with carcinoma of the cervix uteri using step sectioning: Frequency, topographic distribution and prognostic impact. Gynecol Oncol. 2008;111(2):276–81.
55. Cibula D, Abu-Rustum NR, Dusek L, Zikán M, Zaal a SL, et al. Prognostic significance of low volume sentinel lymph node disease in early-stage cervical cancer. Gynecol Oncol. 2012;124(3):496–501.
56. Wang HY, Sun JM, Lu HF, Shi DR, Ou ZL, Cancer RYLFS. Micrometastases detected by cytokeratin 19 expression in sentinel lymph nodes of patients with early cervical. Int J Gynecol Cancer. 2006;16(2):643–8.
57. Di Martino G, Crivellaro C, De Ponti E, et al. Indocyanine green versus radiotracer with or without blue dye for sentinel lymph node mapping in stage >IB1 cervical cancer (>2 cm). J Minim Invasive Gynecol. 2017;24:1–6.
58. Liu KJ, Lv XW, Liu Q, et al. Application of carbon nanoparticles in the laparoscopic sentinel lymph node detection in patients with cervical cancer. Acta Acad Med Sin. 2013;35:150–4.
59. Paredes P, Vidal-Sicart S, Campos F et al.. Role of ICG-Tc99-nanocolloid for sentinel lymph node detection in cervical cancer: a pilot study. Eur J Nucl Med Mol Imaging. 2017;44(11):1853–61.
60. Reade CJ, Eiriksson LR, Covens A. Surgery for early stage cervical cancer: how radical should it be? Gynecol Oncol. 2013;131(1):222–30.

Fertility-Sparing Surgery for Early-Stage Uterine Cervical Cancer

9

Elisa Moreno-Palacios, Claudia Blancafort, Maria Lombarte, and Ignacio Zapardiel

Cervical cancer is the second cause of cancer deaths in women globally, with an estimated 88% of deaths occurring in developing countries [1]. Almost 70% of the global burden occurs in developing countries, where it accounts for almost 12% of all female malignancies, being a major public health problem [2]. It is well known that the most important cause of cervical cancer is the presence of a persistent papillomavirus infection. The risk factors for developing cervical cancer are the same as those for acquiring human papillomavirus (HPV) infection, such as early age intercourse, multiple sexual partners, and sexual contact with high-risk men. HPV type 16 and 18 are responsible for approximately 70–75% of all cervical tumors [3].

However, long-term (1992–2010) cancer incidence trends for all racial and ethnic groups show that cervical cancer has experienced the largest decrease in incidence between women, related mostly to cervical cancer screening programs, with Papanicolau smears and HPV-DNA cervical detection [4]. Cervical cancer screening programs are well established in developed countries, but they are still not well implemented in developing countries, where the vast majority of deaths for cervical cancer occurr occur [1].

Globally approximately 44% of all cervical cancers are diagnosed in early stages. The median age of diagnosis is 48 years, although approximately 45% of cervical cancers are diagnosed in women younger than 40 years [5].

E. Moreno-Palacios
Department of Gynecology, Hospital Universitario La Paz, Madrid, Spain

C. Blancafort
Department of Gynecology, Hospital Universitari Quiron-Dexeus, Barcelona, Spain

M. Lombarte
Gynecologic Oncology Unit, La Paz University Hospital, Madrid, Spain

I. Zapardiel (✉)
Gynecologic Oncology Unit, La Paz University Hospital-IdiPAZ, Madrid, Spain

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_9

Table 9.1 FIGO staging classification of cervical cancer

FIGO stage	Extent of disease
FIGO 0	Carcinoma in situ
FIGO 1	Carcinoma limited to the cervix
IA	Microscopic disease
IA1	Stromal invasion ≤3 mm in depth and lateral spread ≤7 mm
IA2	Stromal invasion >3 mm and ≤ 5 mm, lateral spread ≤7 mm
IB	Macroscopic lesion
IB1	Macroscopic lesion ≤4 cm
IB2	Macroscopic lesion >4 cm
FIGO II	Extension to the uterus/parametria/vagina
IIA	Involvement of the upper two-thirds of the vagina without parametrial invasion
IIA1	Lesion <4 cm greatest diameter
IIA2	Lesion >4 cm greatest diameter
IIB	Involvement of the upper two-thirds of the vagina with parametrial invasion
FIGO III	Extension to the pelvic side wall and/or lower third of the vagina
IIIA	Involvement of the lower third of the vagina
IIIB	Extension to the pelvic side wall and/or hydronephrosis
FIGO IVA	Extension to adjacent organs
FIGO IVB	Distant metastasis

Adapted from Pecorelli [35], with permission

Cervical cancer International Federation of Gynecology and Obstetrics (FIGO) classification stages IA, IB, and IIA are considered early-stage tumors (Table 9.1). Stage IA tumors are defined as invasive carcinomas that present with a stromal invasion of less than 5 mm and an horizontal extension of less than 7 mm. Stage IB tumors are defined as invasive carcinomas limited to the cervix that present with a stromal invasion and an horizontal extension greater than 5 mm and 7 mm, respectively. Stage IIA tumors are defined as invasive carcinomas that invade beyond the uterus, but don't involve the parametrium or the lower third of the vagina.

Lymph node status is not included in the FIGO stage classification, although it is the most important independent prognostic factor in early-stage cervical cancer. If lymph node metastasis is present at the time of diagnosis, the 5-year survival rate drops substantially. In stages IB–IIA, the 5-year survival rate drops from 88% to 95% without lymph node metastasis to 51–78% with lymph node metastasis [6]. Lymph node status ought to be assessed in every patient with early-stage cervical cancer treated surgically.

Radical Trachelectomy

Radical hysterectomy (RH) with pelvic lymphadenectomy has been the gold standard treatment for women with early-stage cervical cancer for decades. Another option of treatment in early-stage cervical cancer is radical radiotherapy that has demonstrated equal oncological results as surgery. However, radical surgery and radiotherapy do not spare fertility, and both methods can lead to sexual dysfunction and decreased quality of life.

In developed countries the extended application of cervical cancer screening tests in the feminine population has caused a decrease in mortality and a higher number of early stage cases diagnosed from more advances to earlier stages of the disease, together with the fact that women tend to delay childbearing at later ages, and that cervical cancer incidence peaks within the third decade of life, there is a group of patients with cervical cancer that have not completed their reproductive desire and want to preserve their fertility. Moreover, a high number of young patients with cervical cancer that are diagnosed in early stages are cured. These facts have led to a gradual abandonment of radical surgical procedures in favor of conservative techniques in an effort to preserve fertility without compromising overall survival. Radical trachelectomy (RT) emerged in an attempt to perform radical surgeries conserving the uterus permitting future pregnancies, without compromising oncological safety.

RT consists in the surgical resection of the cervix, the upper vagina with adequate margins, and 2/3 of the cardinal and uterosacral ligaments. Frozen section of the superior surface of the amputated cervix and endometrial sampling ought to be done to ensure tumor-free margins. The vaginal cuff is anastomosed to the distal part of the uterine corpus once the cervix is excised. A cervical cerclage suture can be placed in the superior margin in theory; it is thought to maintain cervical competence in any future pregnancy. RT is always preceded of pelvic lymphadenectomy, and/or sentinel lymph node biopsy, due to the fact that the presence of metastatic lymph nodes is an absolute contraindication for this procedure.

Dargent et al. was the first to describe vaginal radical trachelectomy (VRT) in early-stage cervical cancer, in 1986. The surgical procedure consisted in the performance of a laparoscopic pelvic lymphadenectomy and vaginal extirpation of the cervix, parametrium, and upper one-third of the vagina [7]. Dargent et al.'s experience was published in 1994, including a total of 28 patients who underwent VRT and pelvic lymphadenectomy. After an average follow-up of 33 months, one patient suffered a distant metastasis. Concerning childbearing, of the 10 patients who desired pregnancy, 8 patients became pregnant, and 11 pregnancies were observed, reporting 3 early spontaneous abortions, 1 legal termination (Down's syndrome), 2 late miscarriage, and 5 deliveries through cesarean section (32,34, 37, 37, and 37 weeks) [8].

In 1997, Smith et al. published the first report of abdominal radical trachelectomy (ART) with reanastomosis of the uterine arteries [9]. Posteriorly in 2005 Smith et al. reported their experience with ART and pelvic lymphadenectomy in 33 patients. Intraoperative complications were rare, and postoperative complications were similar to those of radical hysterectomy. After a median follow-up of 42 months, no recurrences were found. Five patients tried to conceive, resulting in three pregnancies: one miscarriage at 5 weeks and two patients delivered at term by cesarean section [10].

Chyi-Long et al. in 2003 published the first study describing RT by minimal invasive surgery, reporting two cases in which RT was performed entirely by laparoscopy [11]. In 2009, Burnett et al. reported the first case series of robotic radical trachelectomy (RRT), including six women [12]. Burnett et al. described

the surgical robotic procedure emphasizing the advantages of robotics, improved visualization due to three-dimensional optics, improved tissue manipulation due to 360° grades of movement of the instruments, and improved dissection due to the magnification of the surgical field, facilitating the preservation of the superior branches of the uterine artery. Moreover, minimal invasive surgery has shown a decreased tissue trauma and adhesions formation, diminished postoperative pain, less blood loss, shorter hospitalization, and better cosmetic results.

Gradually RT gained worldwide acceptance as a surgical treatment for selected young patients with early-stage cervical cancer who have a strong desire to preserve fertility. Multiple studies comparing oncological results of RH and RT have been published. Beiner et al. reported in 2008 a matched case-control study that included 90 patients submitted to VRT matched to 90 patients in which RH was performed, and no statistical significant difference was found in recurrence rate or overall survival in both groups [13], concluding that VRT is a safe procedure with a recurrence rate of 5%, pointing it out as the procedure of choice in women with small early-stage cervical cancers wishing to preserve fertility. In 2011, Xu et al. published a meta-analysis comparing the clinical effectiveness of RT with RH, including a total of 587 patients, 248 patients in the RT group and 339 in the RH group. The meta-analysis showed that RT had similar efficacy and safety to RH for early cervical cancer, presenting several advantages such as reduced blood loss, shorter time to resumption of urinary function, and shorter postoperative hospital stay [14].

Patient Selection

In order to assure oncological safety, patients for fertility-sparing surgery must be carefully selected. Lymph node metastasis, tumor size, parametrial involvement, stromal invasion, and distance between the tumor and the internal os of the uterus are crucial to take into account to select patients for fertility-preserving surgery.

Lymph Node Status

The first selection criteria is node-negative disease. Patients with nodal spread should be treated with adjuvant chemoradiotherapy, and therefore fertility can't be preserved. The risk of pelvic lymph node metastasis is about 8% in stage IA2, increasing to 15–20% in stage IB1 tumors, and 30% in locally advanced cancer. Stages IB1, with tumors larger than 2 cm, and IB2 are unsuitable for fertility-sparing surgery because the rate of lymph node metastases ranges from 30% to 40%. Nodal dissection ought to be the first surgical step to indicate the fertility-sparing surgery, presenting two alternatives: sentinel node mapping and total pelvic lymphadenectomy [15].

Sentinel lymph node mapping is a useful method for detecting lymph node metastases, although this procedure is still under investigation in cervical cancer. Some studies have shown that sentinel lymph node biopsies may reduce the need of

lymphadenectomy in patients with early-stage cervical cancer. The best mapping results and detection rates are observed in tumors smaller than 2 cm. However, the detection rate and sensitivity remain low in patients with tumors larger than 2 cm and stages IB2 or more. In addition, the efficiency of SLNM diagnosis depends on the mapping method, such as blue dye, radiotracer, or the combination of both, and whether the patient has received neoadjuvant chemotherapy or not.

Furthermore, the accuracy of frozen section in SLN in cervical cancer patients is discussed due to the fact that it presents a limited sensitivity, because of the low rate of diagnosis of micrometastases and isolated tumor cells during intraoperative assessment. Further studies are necessary for SLNM to become a standard procedure.

Tumor Size

The risk of positive lymph nodes, the depth of invasion, and the presence of lymphovascular space invasion (LVSI) increase with the size of the tumor. Park et al. published a study including 1415 patients with early cervical cancer divided into 4 groups based on tumor size: smaller than 2 cm, 2–4 cm, 4–6 cm, and larger than 6 cm. Comparing the groups of tumors smaller than 2 cm with those of 2–4 cm, the authors found significantly lower rates of LVSI (11.4% vs 25.7%), deep stromal invasion (15.7% vs 40.2%), vaginal involvement (5.2% vs 11.2%), parametrial involvement (2.8% vs 12.2%), positive margins (0.9% vs 3.4%), lymph node metastases (6% vs 18.4%), and need for adjuvant therapy (13.6% vs 34%). Preoperative images are crucial to detect major tumor characteristics. MRI is the best technique because it shows tumor size, depth of stromal invasion, and distance to the internal os. Generally, RT is not offered to patients with tumors larger than 2 cm because of the high recurrence rate.

Histological Type

Clear-cell carcinoma and neuroendocrine tumors are absolute contraindication for fertility-sparing surgery, as they are associated with distant metastases and poor oncological outcomes. Adenocarcinoma is suitable for fertility-preserving surgery. Preoperative biopsies provide basic prognosis information including histological type and the presence of LVSI. Adenocarcinomas with severe LVSI present high risk of recurrence, recommending ART [16].

Relative Contraindications

A retrospective study has indicated that stage IB1 cervical cancer should be treated with RH when the cervical tumor is less than 5 mm from the internal os. In this study, preoperative MRI identified those patients who were most likely to benefit

from RH as opposed to RT. Intraoperative assessment of endocervical involvement and tumor-free margins is mandatory, considering 5 mm of the minimum margin oncologically accepted. For fertility results, it is important to consider that the greater volume of cervical stroma conserved, the more chance there is of having a successful pregnancy; the normal recommendation is to preserve 1 cm of healthy tissue [17]. Another prospective study of 30 patients with early-stage cervical cancer treated with RT revealed that MRI had a sensitivity, specificity, and positive predictive value to detect tumor extension beyond the os of 100%, 96%, and 83%, respectively. Thus, measuring the distance between the tumor and internal os by preoperative MRI may indicate whether RT can be attempted.

There are two histological factors that should be taken into account, such as depth of stromal invasion and LVSI. These are prognostic factors for recurrence in early-stage disease, but do not contraindicate fertility-sparing surgery per se.

To ensure the absence of histological invasion of the endocervix, most teams perform intraoperative frozen sections of the surgical margins, and if positive they perform a RH. Some teams also consider stromal invasion deeper than 1 cm, invasion of two-thirds of the cervical stroma, or both to be relative contraindications for fertility-sparing surgery. Tumor grade should probably be considered, but very few recommendations for fertility-sparing surgery strategies include this criteria. The recent discussion for conservative strategies concerns stage IB tumors smaller than 4 cm.

Nonsurgical Strategies for Fertility Preservation in Women with Cervical Cancer

As said before, offering fertility-sparing options – surgical or not – to a woman with gynecological malignancies has become almost mandatory for the scientific community. Some strategies and techniques developed in this emerging field enable the improvement of fertility results after a fertility-spring surgery or can be an option when more advanced disease is found, and there's no apparent way to maintain reproductive health.

Oocyte Cryopreservation

Oocyte cryopreservation has been largely developed during the past years, achieving clinical pregnancy, and live birth rates comparable to those obtained with fresh cycles, and becoming a real alternative to embryo freezing. It constitutes a good strategy for those patients who can postpone up to two weeks the medical or surgical treatment. Nowadays, the use of gonadotropin-releasing hormone antagonists allows a random start of the stimulation at any time of the natural cycle.

Embryo Cryopreservation

This technique is the most standardized procedure of fertility preservation, but the need of a male partner or sperm donor is a requirement that cannot be fulfilled always.

Ovarian Tissue Cryopreservation

Ovarian tissue cryopreservation remains an experimental technique. The ovarian tissue is obtained by laparoscopy and frozen in thin slides. Nevertheless, after the tissue is thawed, and grafted, a massive loss of follicles is suffered. Transplantation can be done in the peritoneal cavity, in the forearm, or in anterior abdominal wall. Due to the lack of worldwide registers, the effectiveness of this technique remains unknown, although it is an option for prepuberal girls or women who cannot undergo an ovarian stimulation or delay their cancer treatment. The main concern is the possibility of reseeding malignant cells. Transplantation of the whole ovary with its vascular pedicle still remains an experimental procedure in humans.

Ovarian Transposition in Cervical Cancer

Since the early 1970s, many investigators have shown that ovarian transposition in women undergoing pelvic radiation may allow preservation of ovarian function. Nowadays, this procedure enables future ovarian stimulation with the production of numerous eggs, which can later be retrieved under ultrasound guidance from the relocation site.

Hormonal Protection by Ovarian Activity Suppression

This technique stands in the idea of maintaining the ovarian metabolism quiescent to avoid any damage caused by oncological treatments but is still controversial.

In Vitro Maturation of Human Oocytes

Retrieving immature oocytes and then performing an oocyte maturation in vitro are other options for those cases in which ovarian stimulation is not possible.

Follicular Culture

This futuristic technique is being proposed as an alternative to ovarian tissue transplantation to avoid the risk of reintroducing malignant cells. Nevertheless, the follicle genesis in vivo is very complicated, so research is still needed.

Follow-Up

Cervical Pap smear after fertility-sparing surgery, especially after trachelectomy, can have normal results interpreted as atypical. Specimens frequently contain glandular cells or endometrial stromal cells from the lower uterine epithelium.

Traditional management based on gynecological and physical examination, and symptom-related discussion, shows very low potential for the detection of recurrent disease. This suggests that patients after fertility-preserving surgery could be managed similarly to patients after excisional treatment for preinvasive cervical lesions.

Early detection of recurrence is an essential condition for favorable prognosis. Colposcopy alone and in combination with Papanicolau test shows a significant sensitivity for the prediction of recurrence.

Obstetrical, Perinatal, and Sexual Outcomes

When analyzing pregnancy outcomes after RT, it is important to take into account that pregnancy rates should be calculated based on the number of patients who attempted pregnancy, and not on the total number of patients preserving fertility. Pregnancies after RT have higher rates of pregnancy lost than the general population (24% for ART, 30% for VRT, and 12% for general population) and preterm delivery caused by a higher risk of premature rupture of membranes due to ascending infection as a result of shortening of the cervix and lack of mucus. After 618 VRT in 10 centers, there were 300 conceptions, 186 deliveries (62%) and 190 babies, 68 first trimester losses (22,7%), and 29 s trimester losses (9,7%) [17].

Up to date, the largest review performed to evaluate fertility and pregnancy outcome is by Bentivegna et al. where the five techniques are evaluated with an overall fertility of 55%, an overall live birth rate of 70%, and an overall prematurity rate of 38% [18] (Table 9.2).

In most series published, around 50% of the patients desiring conception were referred to infertility units. The main issues that compromise fertility are previous infertility, cervical stenosis which occurs in a 40% of the cases but can be solved in approximately 66% [19], lack of cervical mucus, and eventual problems in uterine perfusion after trachelectomy, although there's already evidence assuring its maintenance due to ovarian arteries [20].

The most frequent treatment used is cervical dilatation with or without subsequent in vitro fertilization or intrauterine insemination. After those infertility treatments, between 30% and 80% of the patients seem to achieve pregnancy.

Table 9.2 Obstetrical outcomes

Parameter (rates)	Simple trachelectomy/ conization (%)	Dargent procedure (%)	ART (laparotomy) (%)	ART (laparoscopy) (%)	NACT (%)	Total (%)
Pregnancy	56	57	44	65	77	55
Live birth	74	67	68	78	76	70

Adapted from Bentivegna et al. [18], with permission

Pregnancies achieved after fertility-sparing surgery are always considered high-risk pregnancies, as they are at high risk of preterm delivery, being significantly higher in those patients undergoing ART. Fetal loss is something couples should be advised of, probably due to shorter cervix or incompetence, which leads in higher risk of premature membrane rupture and chorioamnionitis.

There is no consensus on the adequate interval between surgery and conception, but a minimum of 3–6 months seems to be justified. Another concern in pregnancy after a fertility-sparing technique is the eventual need of prophylactic treatment during pregnancy. Some authors defend the use of antibiotics and/or sexual abstinence during the pregnancy to prevent infection during the second trimester. Another option defended by some groups is preventive cerclage during the surgery or later once the patient gets pregnant [21]. No strong evidence on this sense has been published yet.

According to the NCCN guidelines 2016, total hysterectomy after completion of childbearing is at the patient's and surgeon's discretion but is strongly advised in women with continued abnormal Pap smears or chronic persistent HPV infection.

A large cohort of 228 patients with history of cervical cancer published by L Chan et al. in 2015 evaluating sexual satisfaction and quality of life following fertility-sparing surgery compared to hysterectomy concluded that there were no difference in sexual function [22]. Nevertheless, studies evaluating physical and emotional impact on patients after fertility-sparing techniques showed that 30% of these patients were still not sexually active 6 months after surgery because they expressed to be "a little afraid" or "somewhat too very afraid" to have sex [23].

Future Alternatives

Less Radical Fertility-Sparing Surgery

RH and RT are not harmless procedures. The removal of the parametrium is associated with perioperative complications such as blood loss, nerve or ureteral injuries, bladder and bowel dysfunction, fistula formation, and sexual dysfunction, diminishing quality of life. In certain cases of early-stage cervical cancer, the risk of parametrial involvement is low. Retrospective studies have demonstrated that in cervical tumor size of <2 cm, limited depth invasion, no LVI, and negative pelvic lymph nodes, the risk of parametrial involvement is approximately 1% or less [24]. The minimum risk of parametrial involvement in selected patients has led to consider simple hysterectomy, simple trachelectomy, or large conization a possible surgical option, diminishing perioperative morbidity [25]. Ramirez et al. reviewed the studies published on conservative management in patients with early-stage cervical cancer, including a total of 260 patients. Follow-up time varied from 1 to 168 months, finding two relapse and one death, concluding that selected patients with low-risk early-stage cervical tumors may be ideal candidates for conservative surgery [26]. Less radical surgery for selected early-stage tumors is still experimental; multicentric studies are currently being carried out to determinate oncological safety and will provide more concrete evidence on the role of conservative surgery in low-risk tumors.

Neoadjuvant Chemotherapy

It is accepted that RT should not be performed in patients with tumors 2 cm or larger because of the high risk of nodal involvement and the high relapse rate (around 20%) [17]. Furthermore, it is also well known that cervical cancer is chemosensitive to platinum-based drugs. Some groups use neoadjuvant (NAC) regimes to downstage cervical cancer before radical surgery; according to the published data, the response rate of NAC in advanced cervical cancer is between 60% and 95% [27–29]. This made some groups to start to think that, in patients with a strong desire for pregnancy, the use of NAC to downstage the disease and posterior fertility-sparing surgery could be an acceptable option, and many studies have been published in the last years [30].

NAC plus large conization or simple trachelectomy, including pelvic lymphadenectomy, has been studied with promising oncological and obstetrical results and live birth rates. Robova et al. published the fertility outcome after NACT and simple trachelectomy in 28 patients compared with VRT, concluding that pregnancy rates are similar (50% to 52%, respectively), first trimester loss are less frequent (7.7% vs 20%, respectively), and delivery rates are similar (77% vs 70%), but second trimester miscarriage seems to be higher (15% to 3%) [31].

Unfortunately, there are limitations to accept NAC combined with fertility-preserving surgery as a standard of care, such as small series, lack of long-term follow-up assuring similar oncological outcome, and no consensus on optimal chemotherapeutic regimen, concluding that further studies are needed.

Uterine Transplantation

In 2014 and 2015, Brännstrom et al. [32–34] published the first series of uterine transplantation from live donors in nine patients achieving interesting results. Seven patients achieved viable uterus after 12 months, and the first live birth after in vitro fertilization was reported. However, only one out of nine patients had undergone a RH for cervical cancer, and eight patients had congenital uterus absence. Unfortunately, although uterus transplantation itself seems feasible and a very promising futuristic option, its application after locally advanced cervical cancer treatments is a high-risk technique, due to the imperative use of high doses of inmunosuppressive drugs that can increase the risk of cancer recurrence and the fact that eventual vascular abnormalities after radiation would difficult this approach in most of this patients.

Conclusion

In conclusion RT with pelvic lymphadenectomy is currently the standard fertility-preserving procedure in selected patients with early-stage cervical cancer who desire to preserve the uterus, permitting future pregnancies. Fertility-sparing procedures in cervical tumors less than 2 cm in size are considered to be oncologically safe surgical procedures.

References

1. Parkhurst JO, Vulimiri M. Cervical cancer and the global health agenda: insights from multiple policy-analysis frameworks. Glob Public Health. 2013;8(10):1093–108.
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108.
3. Cuschieri KS, Whitley MJ, Cubie HA. Human papillomavirus type specific DNA and RNA persistence – implications for cervical disease progression and monitoring. J Med Virol. 2004;73(1):65–70.
4. Edwards BK, Noone A-M, Mariotto AB, Simard EP, Boscoe FP, Henley SJ, et al. Annual report to the nation on the status of cancer, 1975–2010, featuring prevalence of comorbidity and impact on survival among persons with lung, colorectal, breast, or prostate cancer. Cancer. 2014;120(9):1290–314.
5. Covens A, Rosen B, Murphy J, Laframboise S, AD DP, Lickrish G, Colgan T, Chapman W, Shaw P. Changes in the demographics and perioperative care of stage IA(2)/IB(1) cervical cancer over the past 16 years. Gynecol Oncol. 2001;81(2):133–7.
6. Kim SM, Choi HS, Byun JS. Overall 5-year survival rate and prognostic factors in patients with stage IB and IIA cervical cancer treated by radical hysterectomy and pelvic lymph node dissection. Int J Gynecol Cancer. 2000;10(4):305–12.
7. Dargent D, Burn JL, Roy M, La trachélectomie élargie (T.E.). Une alternative á l'hystérectomie radicale dans le traitment des cancers infiltrants développés sur la face externe du col úterin. J Obstet Gynecol. 1994;2:292–5.
8. Dargent D, Mathevet P. Schauta's vaginal hysterectomy combined with laparoscopic lymphadenectomy. Baillière's Clin Obstet Gynaecol. 1995;9(4):691–705.
9. Smith JR, Boyle DC, Corless DJ, Ungar L, Lawson AD, Del Priore G, et al. Abdominal radical trachelectomy: a new surgical technique for the conservative management of cervical carcinoma. Br J Obstet Gynaecol. 1997;104(10):1196–200.
10. Ungár L, Pálfalvi L, Hogg R, Siklós P, Boyle DCM, Del Priore G, et al. Abdominal radical trachelectomy: a fertility-preserving option for women with early cervical cancer. BJOG. 2005;112(3):366–9.
11. Lee CL, Huang KG, Wang CJ, Yen CF, Lai CH. Laparoscopic radical trachelectomy for stage Ib1 cervical cancer. J Am Assoc Gynecol Laparosc. 2003;10(1):111–5.
12. Burnett AF, Stone PJ, Duckworth LA, Roman JJ. Robotic radical trachelectomy for preservation of fertility in early cervical cancer : case series and description of technique. J Minim Invasive Gynecol. 2009;16(5):569–72.
13. Beiner ME, Hauspy J, Rosen B, Murphy J, et al. Radical vaginal trachelectomy vs. radical hysterectomy for small early stage cervical cancer (CC): a matched case-control study. Gynecol Oncol. 2008;110(2):168–71.
14. Xu L, Sun F-Q, Wang Z-H. Radical trachelectomy versus radical hysterectomy for the treatment of early cervical cancer (CC): a systematic review. Acta Obstet Gynecol Scand. 2011;90(11):1200–9.
15. Bentivegna E, Gouy S, Maulard A, Chargari C, Leary A, Morice P. Oncological outcomes after fertility-sparing surgery for cervical cancer (CC): a systematic review. Lancet Oncol. 2016;17(6):e240–53. https://doi.org/10.1016/S1470-2045(16)30032-8.
16. Sato S, Itamochi H, Sugiyama T. Fertility-sparing surgery for uterine cervical cancer. Future Oncol. 2016;12(20):2345–55. https://doi.org/10.2217/fon-2016-0260.
17. Rob L, Skapa P, Robova H. Fertility-sparing surgery in patients with cervical cancer. Lancet Oncol. 2011;12(2):192–200.
18. Bentivegna E, Maulard A, Pautier P, Chargari C, Gouy S, Morice P. Fertility results and pregnancy outcomes after conservative treatment of cervical cancer (CC): a systematic review of the literature. Fertil Steril. 2016;106(5):1195–211.
19. Carter J, Sonoda Y, Chi DS, Raviv L, Abu-Rustum NR. Radical trachelectomy for cervical cancer (CC): postoperative physical and emotional adjustment concerns. Gynecol Oncol. 2008;111(1):151–7.

20. Abbara S, Nikolic B, Pelage JP, Banovac F, Spies JB. Frequency and extent of uterine perfusion via ovarian arteries observed during uterine artery embolization for leiomyomas. AJR Am J Roentgenol. 2007;188(6):1558–63.
21. Halaska MJ, Robova H, Pluta M, Rob L. The role of trachelectomy in cervical cancer. E Cancer Med Sci. 2015;9:506.
22. Chan J, Letourneau J, Salem W, Pelin Cil A, Chan S, Chen L, Rosen M. Sexual satisfaction and quality of life in survivors of localized cervical and ovarian cancers following fertility-sparing surgery. Original Research Article. Gynecol Oncol. 2015;139(1):141–7.
23. Carter J, Sonoda Y, Chi DS, Raviv L, Abu-Rustum NR. Radical trachelectomy for cervical cancer (CC): postoperative physical and emotional adjustment concerns. Gynecol Oncol. 2008;111:151–7.
24. Pluta M, Rob L, Charvat M, Chmel R, Halaska M, Skapa P, et al. Less radical surgery than radical hysterectomy in early stage cervical cancer – a pilot study. Gynecol Oncol. 2009;113(2):181–4.
25. Reade CJ, Eiriksson LR, Covens A. Surgery for early stage cervical cancer (CC): how radical should it be? Gynecol Oncol. 2013;131(1):222–30.
26. Ramirez PT, Pareja R, Rendón GJ, Millan C, Frumovitz M, Schmeler KM. Management of low-risk early-stage cervical cancer (CC): should conization, simple trachelectomy, or simple hysterectomy replace radical surgery as the new standard of care? Gynecol Oncol. 2014;132(1):254–9.
27. Robova H, Halaska M, Pluta M, Skapa P, Strnad P, Lisy J, et al. The role of neoadjuvant chemotherapy and surgery in cervical cancer. Int J Gynecol Cancer. 2010;20:S42–6.
28. Robova H, Rob L, Halaska MJ, Pluta M, Skapa P, Strnad P, et al. High-dose density neoadjuvant chemotherapy in bulky IB cervical cancer. Gynecol Oncol. 2013;128:49–53.
29. Benedetti-Panici P, Greggi S, Colombo A, Amoroso M, Smaniotto D, Giannarelli D, et al. Neoadjuvant chemotherapy and radical surgery versus exclusive radiotherapy in locally advanced squamous cell cervical cancer (CC): result from the Italian multicenter randomized study. J Clin Oncol. 2002;20(1):179–88.
30. Tomao F, Corrado G, Peccatori FA, Boveri S, Preti E, Colombo N, Landoni F. Fertility-sparing options in young women with cervical cancer. Curr Treat Options Oncol. 2016;17(1):5. https://doi.org/10.1007/s11864-015-0386-9.
31. Robova H, Halaska M, Pluta M, Skapa P, Matecha J, Lisy J, Rob L. Oncological and pregnancy outcomes after high-dose density neoadjuvant chemotherapy and fertility-sparing surgery in cervical cancer. Original Research Article. Gynecol Oncol. 2014;135(2):213–6.
32. Brännström M, Dahm-Kähler P, Enskog A, Johannesson L, Lundmark C, Olausson M, et al. Transplantation of the uterus still at the experimental stage. Lakartidningen. 2014;111(18–19):806–7.
33. Johannesson L, Kvarnström N, Mölne J, Dahm-Kähler P, Enskog A, Diaz-Garcia C, et al. Uterus transplantation trial: 1-year outcome. Fertil Steril. 2015;103(1):199–204.
34. Brännström M, Johannesson L, Bokström H, Kvarnström N, Mölne J, Dahm-Kähler P, et al. Livebirth after uterus transplantation. Lancet. 2015;385(9968):607–16.
35. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet. 2009;105(2):103–4.

Standard and Novel Surgical Treatment in Cervical Cancer

10

Georgios Androutsopoulos and Raj Naik

Cervical cancer (CC) represents a major clinical problem as it is globally the most common malignancy of the female reproductive system with high incidence among young women [1, 2]. The mortality rate is significantly higher in developing regions (Melanesia, Africa, South-Central Asia, Caribbean, and South America), when compared with more developed ones (Australia, New Zealand, West and North Europe, West Asia, and North America) [1, 2].

Based on recently published guidelines, the primary management of CC mainly depends on disease stage and could be either surgical, nonsurgical (radiotherapy, chemoradiotherapy), or a combination of both [3–5]. However, treatment planning should be made by a multidisciplinary team (MDT), including gynecological oncologist, radiation oncologist, medical oncologist, pathologist, radiologist, clinical nurse specialist, and other healthcare professionals.

In the MDT meeting, all available treatment options should be considered, and the final decision regarding the type and extent of surgical treatment should be carefully individualized according to disease stage, histologic subtype, fertility issues, and performance status [3, 5–7].

Conservative Surgical Management

Conservative surgical management is mainly used in CC patients with early-stage disease and desire for fertility preservation.

G. Androutsopoulos (✉)
Department of Obstetrics and Gynaecology, University of Patras, Rion, Achaia, Greece
e-mail: androutsopoulos@upatras.gr

R. Naik
Department of Gynaecology, Northern Gynaecological Oncology Centre, Queen Elizabeth Hospital, Gateshead, UK

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_10

Cervical Conization

The procedure of cervical conization is characterized by the removal of a cone-shaped portion of the cervix [8]. It has dual diagnostic and therapeutic role [8].

Patient Selection

Cervical conization is mainly indicated for young CC patients with FIGO stage IA1 disease with no lymphovascular space invasion (LVSI) and strong desire for fertility preservation [3, 5, 6]. Moreover, CC patients with FIGO stage IA1 and LVSI or IA2 disease are also eligible for conservative management with cervical conization [3, 5, 6].

Additionally, there is some evidence that selected CC patients with FIGO stage IB1 small-volume disease (less than 500 mm^3) could also be treated with cervical conization and pelvic lymph node dissection [9, 10]. These patients should have examination under anesthesia as well as investigation with cystoscopy and pelvic magnetic resonance imaging (MRI), in order to assess the size and extent of disease [10].

The selected patients shouldn't have any metastasis to pelvic and para-aortic lymph nodes [3, 6]. Furthermore, they should have a detailed preoperative assessment as well as extensive counseling regarding disease recurrence, fertility issues, and pregnancy outcomes [3, 6, 7, 11].

Patients with aggressive (small cell neuroendocrine carcinoma) or potentially aggressive (gastric-type adenocarcinoma, minimal deviation adenocarcinoma) histologic subtypes of CC shouldn't be treated with cervical conization even at early-stage disease [3, 12, 13]. Furthermore, the procedure should be avoided in CC patients with positive lymph nodes [3, 6].

Technique Description

The procedure of cervical conization involves the en bloc resection of both ectocervix and endocervical canal in a single specimen and could be performed either with cold knife, laser, or electrosurgery [3, 7, 8, 14]. Cold knife conization is the most preferable approach, because it provides an intact specimen without any thermal effect [3, 15, 16]. This is of great importance, especially in accurate evaluation of the resection margins [3, 7, 15, 16]. In case of loop electrosurgical excision procedure (LEEP), we should take additional care in order to minimize the thermal effect on conization specimen [3, 15, 17].

Especially in CC patients with FIGO stage IA2 with LVSI and IB1 small-volume disease, we should perform pelvic lymphadenectomy with or without sentinel lymph node (SLN) mapping, as the risk of lymphatic metastasis or recurrent disease is significant (Fig. 10.1) [3, 6, 8–10, 18]. In this case, pelvic lymph nodes could be excised laparoscopically [3, 6, 8, 18].

The provided surgical specimen should have at least 3 mm clear margins for preinvasive or invasive disease [3]. In case of positive cone margins, we should either repeat cervical conization or perform radical trachelectomy (Fig. 10.2) [3]. Moreover, in case of positive lymph nodes, then either radical hysterectomy or primary chemoradiotherapy should be performed (Fig. 10.2) [3].

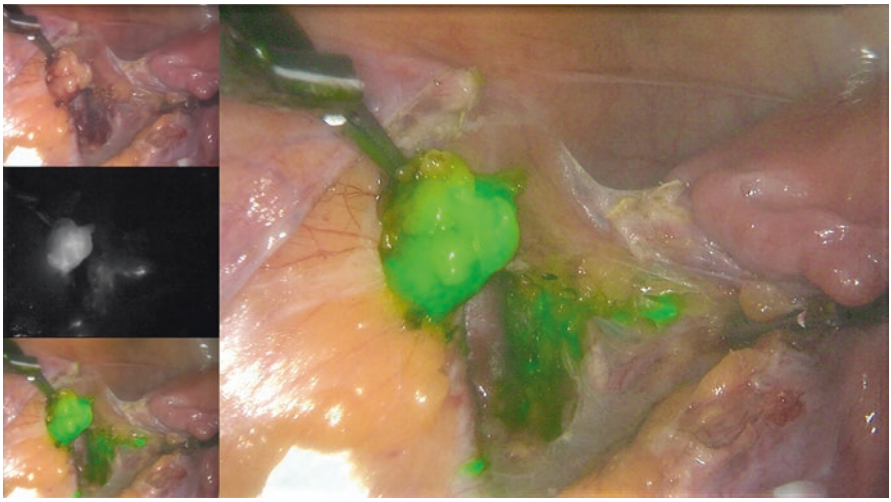

Fig. 10.1 Laparoscopic sentinel lymph node dissection using indocyanine green technique (Novadaq – Pinpoint) [Northern Gynaecological Oncology Centre (NGOC)]

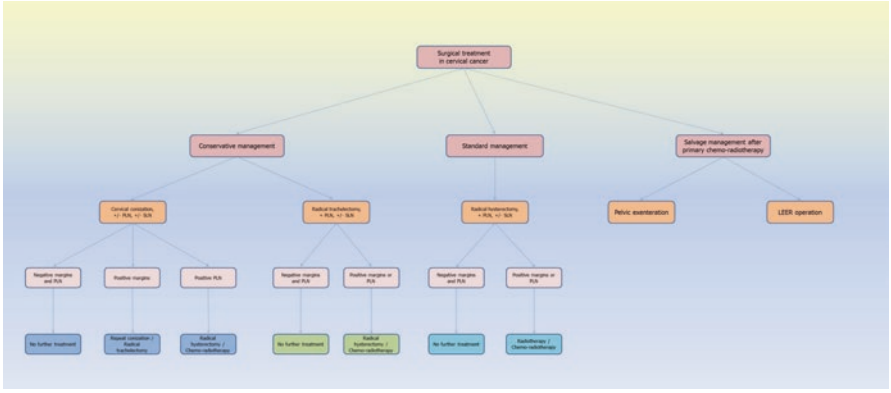

Fig. 10.2 Surgical treatment in cervical cancer. *PLN* pelvic lymph nodes, *SLN* sentinel lymph nodes

Complications

The perioperative morbidity in CC patients treated with cervical conization is relatively low, as it is a conservative therapeutic approach [14].

The most common early postoperative complications in CC patients treated with cervical conization are bleeding, discharge, and wound infection [14]. Similarly, the most common late postoperative complications in these patients are cervical stenosis and impaired cervical function [14, 19].

Oncologic Outcome

Overall, cervical conization using either cold knife or electrosurgery represents a feasible treatment option for young CC patients with FIGO stage IA1 and IA2 disease [3, 20, 21]. This is mainly because of the fact that the risk of recurrence at this stage disease is relatively low [3, 20, 21].

Especially in CC patients with FIGO stage IA1 disease treated with cervical conization, the recurrence and death rate is about 2.2% and 0.7%, respectively, in case of negative cone margins [22].

The existence of lymph node metastasis, involved resection margins, and LVSI in CC patients treated with cervical conization are poor prognostic factors and associated with increased risk of recurrence [3, 20, 23].

Fertility and Pregnancy Issues

Patients treated with cervical conization that is greater than 1 mm deep have impaired cervical function and pregnancy complications, despite the technique used [19, 24, 25]. The number of conizations, as well as the depth and volume of excised cervical tissue, is associated with increased risk of preterm labor [19, 24, 25].

More specifically, the relative risk for preterm labor (<37 weeks) in CC patients treated with cold knife conization is 2.70 (95% CI 2.14–3.40) while in others treated with laser conization or LEEP is 2.11 (95% CI 1.26–3.54) and 1.56 (95% CI 1.36–1.79), respectively [24]. Furthermore, the relative risk for premature rupture of membranes (<37 weeks) in CC patients treated with cold knife conization is 4.11 (95% CI 2.05–8.25) while in CC patients treated with LEEP is 2.15 (95% CI 1.48–3.12) [24].

Overall, LEEP and laser provide better pregnancy outcomes, when compared with cold knife conization [19, 25]. This is the main reason why electrosurgery and laser represent the most preferable techniques for cervical conization.

Radical Trachelectomy with Lymph Node Dissection

The procedure of radical trachelectomy was initially described by Daniel Dargent in 1994, for the fertility-sparing treatment of young patients with early-stage CC [26, 27]. In 1997, Richard Smith proposed abdominal radical trachelectomy, as an alternative to the vaginal approach [28].

Patient Selection

Radical trachelectomy is mainly indicated for young CC patients with FIGO stage IB1 disease and strong desire for fertility preservation [3, 5, 27, 29, 30]. Preoperatively, all patients should have a pelvic MRI and examination under anesthesia +/− investigation with cystoscopy, in order to assess tumor size, extent, and proximity to internal cervical os [3, 5, 31–33].

The eligible patients should have tumor size less than 2 cm in greatest dimension and no involvement of the upper endocervical canal [3, 5, 29, 30, 34–36]. Furthermore, they shouldn't have any metastasis to pelvic and para-aortic lymph nodes [27, 29, 30, 36]. In addition, they should have detailed counseling about disease recurrence, fertility issues, pregnancy, and perinatal outcomes [3, 6, 7, 11, 30, 36–38].

Patients with aggressive (small cell neuroendocrine carcinoma) or potentially aggressive (gastric-type adenocarcinoma, minimal deviation adenocarcinoma) histologic subtypes of CC are not eligible for radical trachelectomy even at early-stage disease [3, 12, 13, 29, 39].

Technique Description

There are two main types of radical trachelectomy: vaginal and abdominal [27, 28, 40]. Pelvic lymphadenectomy with or without SLN mapping should be performed before starting the trachelectomy (Fig. 10.1) [27, 40, 41]. The excised lymph nodes should be examined with multiple frozen sections, and in case of positive lymph nodes, the procedure should be abandoned [27, 40–42].

Vaginal radical trachelectomy involves the en bloc resection of cervix, upper vagina, and paracolpos and paravaginal tissues, as in a type B radical hysterectomy [3, 27]. In these patients, pelvic lymph nodes should be excised laparoscopically [27]. On the other hand, abdominal radical trachelectomy involves the en bloc resection of the cervix, upper vagina (1–2 cm), parametrium, and paracolpos/paravaginal tissues, as in a type C radical hysterectomy (Figs. 10.3 and 10.4) [3, 28, 30, 40]. The procedure provides a wider parametrial resection and represents a less conservative approach, when compared with vaginal radical trachelectomy [3, 28, 30, 34, 35, 40, 43]. It could be performed either with the standard or the minimally invasive approach (laparoscopy, robotic-assisted surgery), with no evidence of compromise in oncologic management [3, 44–47]. Minimally invasive approach provides many advantages in intraoperative blood loss, hospitalization, and postoperative recovery [44].

The provided surgical specimen should be examined with frozen sections, in order to clarify the status of resection margins (Fig. 10.4) [42, 48]. If frozen sections are negative with clear margins to the endocervix, then radical trachelectomy has already completed (Fig. 10.2) [30, 40, 48]. If there is residual tumor on the specimen margins or positive lymph nodes, then either radical hysterectomy or primary chemoradiotherapy should be performed (Fig. 10.2) [29, 39, 40, 42, 48–50].

Before uterine reconstruction, a permanent cervical cerclage is placed around the isthmus in order to reduce the future risk of preterm labor [29, 30, 41, 42, 44, 48]. Subsequently, the cervical stump should be re-sutured to the vaginal cuff [29, 30, 41, 42, 44, 48].

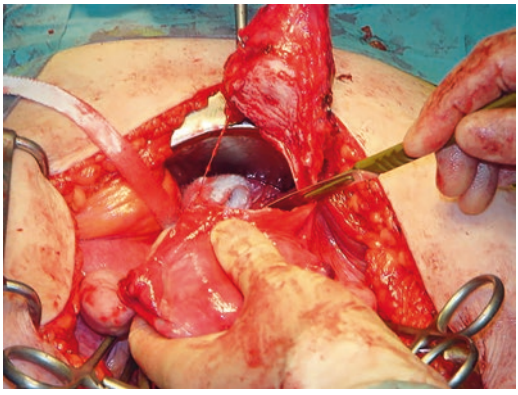

Fig. 10.3 Radical abdominal trachelectomy – surgical technique [Northern Gynaecological Oncology Centre (NGOC)]

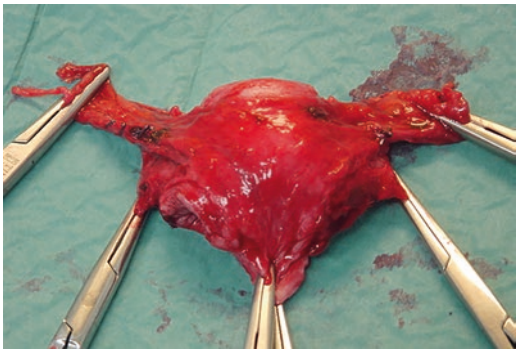

Fig. 10.4 Radical abdominal trachelectomy – surgical specimen [Northern Gynaecological Oncology Centre (NGOC)]

Complications

The perioperative morbidity in CC patients treated either with vaginal or abdominal radical trachelectomy is quite acceptable for a radical procedure [29, 42, 43, 51]. Moreover, patients treated with radical trachelectomy have less significant perioperative complications, when compared with radical hysterectomy [52].

The rate of intraoperative complications is significantly higher for vaginal (5.6%), when compared with abdominal radical trachelectomy (0.7%) [29, 42, 43, 51, 53]. In contrast, the rate of postoperative complications is significantly greater for abdominal (35%), in comparison with vaginal radical trachelectomy (7.5%) [29, 42, 51, 53]. This is mainly because of the fact that abdominal approach is a more extensive surgical procedure providing a wider parametrial resection and the need for an abdominal incision [29, 42, 43, 51, 53].

The most common early postoperative complications in patients after radical trachelectomy are bladder hypotonia, urinary tract infection, deep venous thrombosis, pulmonary embolism, lymphatic cyst, and genitofemoral nerve palsy [29, 39, 42, 44, 51, 53]. Similarly, the most common late postoperative complications in these patients are cervical stenosis, chronic pelvic pain, and lymphoedema [29, 39, 51, 53].

Oncologic Outcome

Both types of radical trachelectomy provide similar oncologic outcomes, despite a wider parametrial resection when using the abdominal approach [43]. The recurrence rate in CC patients treated with abdominal or vaginal radical trachelectomy is about 3.8% and 4.8%, respectively [29, 34, 35, 39, 42, 51, 53–55]. Moreover the mortality rate in CC patients treated with radical trachelectomy is about 0.4% for the abdominal and 2.9% for the vaginal approach [29, 34, 35, 39, 42, 51, 53–55].

The overall and disease-free survival rate at 5 years in CC patients treated with vaginal radical trachelectomy is about 97% and 95%, respectively [34, 42, 51, 54, 55]. Moreover, the disease-free survival rate at 5 years in CC patients with stage IB1 disease is almost equal between those having radical trachelectomy and radical hysterectomy [34].

The tumor size has a direct correlation with oncologic outcome [29, 50]. Especially in CC patients with tumor size more than 2 cm treated with vaginal radical trachelectomy, the risk of disease recurrence is more than 12% (range 12–29%) [27, 29, 35, 36, 42, 50, 51, 56]. This is the main reason why vaginal radical trachelectomy should be avoided in this patient subgroup [29, 42, 50, 51, 56]. However, recent studies evaluating the role of abdominal radical trachelectomy in CC patients with tumor size between 2 and 4 cm have shown very promising results [35, 49, 56]. This is mainly due to the wider resection of parametrial tissue [35, 40, 43].

Fertility and Pregnancy Issues

Patients treated with vaginal radical trachelectomy have more favorable fertility outcomes, when compared with patients having the abdominal approach [29, 35, 42]. More specifically, the pregnancy rate in CC patients treated with radical trachelectomy is about 16% for the abdominal and 55% for the vaginal approach [29, 35, 42, 53, 57]. This is mainly because abdominal radical trachelectomy is a less conservative surgical approach [29, 30, 34, 35, 43, 55].

Furthermore, the impaired cervical function increases the risk of miscarriages and preterm labor in all patients treated with radical trachelectomy [3, 29, 35, 42, 57–59]. The rate of first-trimester miscarriages in CC patients having abdominal and vaginal radical trachelectomy is about 12% and 20%, respectively [39, 42, 53, 54, 57]. The rate of second-trimester miscarriages is significantly greater in CC patients treated with abdominal radical trachelectomy (12%), compared with others having vaginal radical trachelectomy (3%) [39, 42, 53, 57]. Additionally, the rate of preterm labor in CC patients having radical trachelectomy is similar after either the abdominal (16%) or vaginal approach (18%) [42, 53].

The mode of delivery after radical trachelectomy should be an elective cesarean section at 38 weeks of gestation [42]. This is because of the previous operation and the increased risk of uncontrolled cervical injuries during vaginal delivery [42].

Standard Surgical Management

Standard surgical management is mainly used in CC patients with early-stage disease, who have already completed their childbearing.

Radical Hysterectomy

The procedure of radical hysterectomy was initially described by Ernst Wertheim in 1898, for the treatment of CC [60]. In 1901, Friedrich Schauta proposed vaginal radical hysterectomy, as an alternative to the abdominal approach [61]. In 1995, Daniel Dargent modified the Schauta operation and included laparoscopic lymphadenectomy (Celio-Schauta procedure) [61].

Patient Selection

Radical hysterectomy is mainly indicated for CC patients with FIGO stage IB or IIA disease, when fertility preservation is not desired [3, 5, 6, 62]. Furthermore, radical hysterectomy may be considered in highly selected CC patients with persistent or recurrent disease <2 cm confined to the central pelvis, who have already been treated with radiotherapy or chemoradiotherapy [3, 63].

Preoperatively, all patients should have examination under anesthesia and investigation with cystoscopy and pelvic MRI, in order to assess the size and extent of disease [3, 5, 32]. Whole-body positron emission tomography (PET-CT) or chest, abdomen, and pelvic computed tomography (CT) could also be considered [3, 5].

CC patients with FIGO stage IB2 or IIA2 disease and bulky tumors shouldn't be treated with radical hysterectomy, as there is an increased need for adjuvant radiotherapy in these cases resulting in the additional morbidity associated with dual modality therapy. Chemoradiotherapy is a more effective therapeutic approach and remains the treatment of choice [3, 6, 64–67]. Alternatively, one could consider laparoscopic lymphadenectomy in these cases as an initial staging procedure and carry out a radical hysterectomy as a second procedure in the node-negative cases only [68]. CC patients with advanced-stage disease (FIGO stage IIB to IVA) as well as others with distant metastases (FIGO stage IVB) are not eligible for radical hysterectomy [3, 6, 64, 66, 69, 70].

There is some evidence that radical hysterectomy could be avoided in selected CC patients with FIGO stage IB1 small-volume disease (less than 2 cm), as cervical conization or simple hysterectomy with pelvic lymph node dissection provides equal oncologic result [9, 10]. This is mainly based on the fact that CC patients with FIGO stage IB1 disease and negative pelvic lymph nodes have less than 2% parametrial involvement [10, 71]. However, this approach remains under evaluation with an ongoing prospective randomized trial (ConCerv trial [NCT01048853], SHAPE trial [NCT01658930]) [72, 73].

Technique Description

There are two main types of radical hysterectomy: vaginal and abdominal [41]. Pelvic lymphadenectomy with or without SLN mapping should be performed in all patients [3, 41, 62, 64, 74]. However, the role of intraoperative assessment of lymph node status with multiple frozen sections in order to perform or abandon radical hysterectomy remains controversial [3, 75].

Vaginal and abdominal radical hysterectomy involves the en bloc resection of the uterus, upper vagina, and parametrium and paracolpos/paravaginal tissues [36, 41, 61]. In the vaginal procedures, pelvic lymph nodes should be excised laparoscopically [36, 41, 61]. The abdominal procedure could be performed either with the standard open or the minimally invasive approach (laparoscopy, robotic-assisted surgery), with no evidence of compromise in oncologic outcome [3, 36, 76–79]. Minimally invasive approach provides many advantages in intraoperative blood loss, hospitalization, postoperative pain, and recovery, while there are no significant differences in the risk of perioperative complications [76–78]. However, this approach requires special training and specific surgical skills [76, 78].

Depending on the lateral extent of surgical resection, the radical hysterectomy procedure is classified into type A (minimum resection of paracervix), type B (transection of paracervix at the level of ureteral tunnel), type C (transection of paracervix at junction with internal iliac vascular system), and type D (laterally extended resection) [80, 81].

The type B procedure corresponds to a modified radical hysterectomy [80, 81]. In this procedure, the paracervix should be transected at the level of ureteral tunnel [80]. In addition, the utero-sacral and vesico-uterine ligaments should be excised partially with at least 10 mm of the vagina [80].

The type C procedure corresponds to a classical radical hysterectomy [80, 81]. In this procedure, the paracervix should be transected at the junction with the internal iliac vascular structures [80]. In addition, the utero-sacral and vesico-uterine ligaments should be excised completely along with 15–20 mm of the vagina and paracolpos (Fig. 10.5) [80]. Preservation of the hypogastric nerve plexus results in further subclassification: type C1 (with nerve preservation) and type C2 (without nerve preservation) [80, 81].

Regarding lymph node dissection, this can also be classified into four different levels according to arterial anatomy: level 1 (external and internal iliac), level 2 (common iliac and presacral), level 3 (aortic infra-mesenteric), and level 4 (aortic infrarenal) [80].

Recently, SLN mapping and dissection have gained popularity especially in CC patients with early-stage disease (see Fig. 10.1) [7, 36, 82, 83]. The procedure of SLN mapping and dissection decreases significantly the morbidity of systematic lymphadenectomy, without affecting survival [7, 36, 82, 83]. Moreover, the utilization of ultrastaging in dissected lymph nodes plays a crucial role in detection of micrometastases [82, 84].

According to the SLN surgical algorithm, any suspicious or enlarged lymph node should also be removed, apart from the SLNs [82]. In case of failed SLN mapping, a side-specific pelvic lymph node dissection should be performed [82]. All excised SLNs should be evaluated by the pathologist using ultrastaging [82].

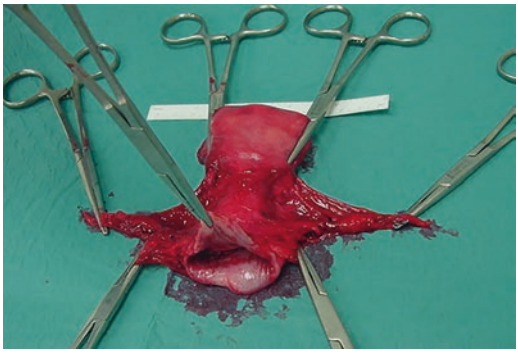

Fig. 10.5 Radical abdominal hysterectomy – surgical specimen [Northern Gynaecological Oncology Centre (NGOC)]

Complications

The perioperative morbidity in CC patients treated either with modified or classical radical hysterectomy is quite similar, with an average rate of 5% and 4%, respectively [74]. However, late postoperative morbidity and especially bladder and anorectal function are mainly affected by the extent of parametria resection and nerve preservation in CC patients having radical hysterectomy [36, 74, 81, 85, 86]. This is the main reason why the average rate of late postoperative complications is significantly higher in CC patients treated with type C radical hysterectomy (38%), when compared with others treated with type B operation (28%) [74, 85].

The most common early postoperative complications in CC patients treated with radical hysterectomy are deep venous thrombosis, pulmonary embolism, bowel obstruction, pelvic abscess, sepsis, uretero-vaginal fistula, vesico-vaginal fistula, lymphatic cyst, and peripheral nerve injury [41, 74, 86]. Likewise, the most common late postoperative complications in these patients are bladder dysfunction, anorectal dysfunction, bowel obstruction, uretero-vaginal fistula, vesico-vaginal fistula, and lymphoedema [41, 74, 85, 86].

Oncologic Outcome

The oncologic outcome in CC patients treated either with type B or type C radical hysterectomy is quite similar, despite differences in the extent of parametria resection [74, 86]. The recurrence rate in CC patients treated either with type B or type C radical hysterectomy is about 24% and 26%, respectively [74]. In addition, the mortality rate in CC patients is about 18% for those having a type B and 20% for others having a type C radical hysterectomy [74].

The overall and disease-free survival rate at 5 years in CC patients treated with type B radical hysterectomy is about 81% and 75%, respectively [74]. Similarly, the overall and disease-free survival rate at 5 years in CC patients treated with type C radical hysterectomy is about 77% and 73%, respectively [74].

Patients with two or more of the following features, >1/3 stromal invasion, LVSI, and tumor diameter > 4 cm (Sedlis criteria), have increased risk of disease recurrence [3, 5, 68, 74, 87]. In addition, the presence of lymph node metastasis or involved resection margins in CC patients treated with radical hysterectomy are also high-risk factors for disease recurrence [3, 5, 68, 74, 87]. Consequently, we should consider postoperative adjuvant radiotherapy or chemoradiotherapy in CC patients with poor prognostic factors, in order to reduce the risk of disease recurrence and improve the oncologic outcome (see Fig. 10.2) [3, 5, 74, 87–90].

Salvage Surgical Management

Salvage surgical management is predominantly used in CC patients with locally advanced, persistent, or recurrent disease, who have already treated with radiotherapy or chemoradiotherapy (see Fig. 10.2).

Pelvic Exenteration

The procedure of pelvic exenteration was initially described by Alexander Brunschwig in 1948, for the palliative treatment of advanced pelvic cancer [91]. It is characterized by the en bloc resection of all pelvic viscera and represents an ultra-radical surgical procedure [91].

Patient Selection

Pelvic exenteration is mainly indicated for patients with persistent or recurrent CC confined to the central pelvis, who have already been treated with radiotherapy or chemoradiotherapy [3, 5, 36, 91–101]. The procedure could also be used in patients with locally advanced CC, who have previously received pelvic radiotherapy for another malignancy before developing CC [3, 94]. Moreover, in CC patients with stage IVA disease and without any previous treatment, pelvic exenteration represents an alternative option to chemoradiotherapy [96, 97, 102]. However, pelvic exenteration could possibly be considered as palliative treatment in carefully selected CC patients with vesico-vaginal or recto-vaginal fistula, in order to reduce their symptoms and improve their quality of life [36, 91, 94–97, 103, 104].

Preoperatively, all patients should have examination under anesthesia and investigation with abdomen and pelvic MRI and whole-body PET-CT [94, 95]. Moreover, a histologic confirmation of disease recurrence should also be available [94, 95].

Tumor size, histologic type, and recurrence-free interval from any previous treatment should be considered thoroughly during the selection process [92]. Eligible patients should have completely excisable central pelvic disease [92, 96, 99, 102]. Furthermore, their performance status should be appropriate for an extensive operation [97, 103].

Pelvic exenteration is contraindicated for CC patients with extra-pelvic disease, including para-aortic lymph node involvement and peritoneal or distant metastases [3, 36, 92–99, 102]. In addition, CC patients with pelvic sidewall involvement or with parietal involvement of the sciatic foramen are not eligible for the typical pelvic exenteration [92–96, 101, 102]. In patients with obstructive uropathy, leg lymphedema, or sciatic nerve pain, any pelvic exenterative procedure should be reconsidered as these symptoms may suggest pelvic sidewall involvement [94, 95, 97, 102].

Technique Description

Pelvic exenteration represents a curative approach in CC patients, although it was initially described as a palliative procedure [91–93, 100]. The main aim of this operation is the complete tumor resection with clear margins for tumor [92].

There are three main types of pelvic exenteration: anterior, posterior, and total [94, 95, 105]. Anterior pelvic exenteration involves the en bloc resection of the bladder, distal ureters, and genital organs with their ligamentous attachments and pelvic lymph nodes [91, 93, 94, 98]. Likewise, posterior pelvic exenteration includes the en bloc resection of the genital organs with the rectum and rectosigmoid with ligamentous attachments and pelvic lymph nodes [91, 93, 94, 98]. Total pelvic exenteration

involves the en bloc resection of all pelvic organs with pelvic lymph nodes (Figs. 10.6 and 10.7) [91, 93, 94, 98]. Anterior and total are the most commonly used types in gynecological oncology, while posterior pelvic exenteration is mainly used by colorectal surgeons for locally advanced lower GI tumors [94, 95, 98, 106].

Pelvic exenteration can be further subclassified into type I (supralevator), type II (infralevator), and type III (infralevator with vulvectomy) [92, 105]. Depending on tumor location and extent in select cases, the pelvic floor and anal sphincter could possibly be preserved by performing a type I pelvic exenteration (Figs. 10.6 and 10.7) [3, 92, 95, 105]. Otherwise, the lower vagina and rectum should be excised by performing a type II or type III pelvic exenteration in order to obtain clear resection margins [3, 92, 94, 105]. However, type III pelvic exenteration is usually performed in patients with a tumor size more than 5 cm or with direct extension to the lower part of the vagina, the anal canal, and the vulva [92, 105].

The provided surgical specimen should be examined with frozen sections, in order to clarify margin and lymph node status [3]. If specimen margins are clear, then the excisional part of pelvic exenteration has been completed [3]. Subsequently, surgical reconstruction of the excised pelvic floor as well as urinary and fecal diversion is required [92, 94, 95, 99].

Urinary diversion represents an integral part of an anterior or total pelvic exenteration [107]. Among various types of urinary diversion, ileal conduit and orthotopic neobladder are the most commonly used procedures [107]. Both of them provide similar oncologic results in terms of local recurrence or distant metastasis [107]. However in daily practice, ileal conduit represents an easy, reliable, and less time-consuming procedure, which can be performed by an experienced gynecological oncologist [94, 98, 101, 108, 109]. The uretero-enteric anastomosis in an ileal conduit is usually performed using either a Bricker or Wallace I and II techniques [110–114].

Regarding fecal diversion, a left iliac fossa colostomy should be performed [92, 94, 101]. Due to the prior radiotherapy treatment, any direct anastomosis should be avoided, as it is associated with many postoperative complications and increased risk of early recurrence at the site of colorectal anastomosis [92, 100].

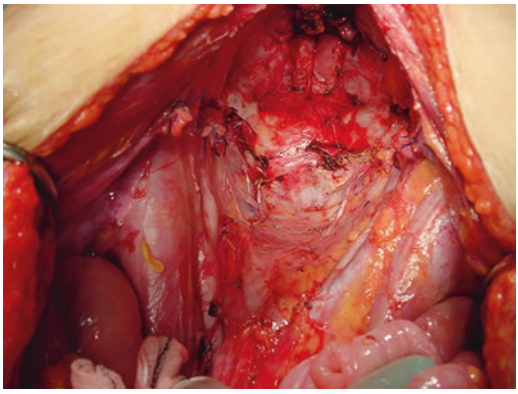

Fig. 10.6 Total supralevator pelvic exenteration [Northern Gynaecological Oncology Centre (NGOC)]

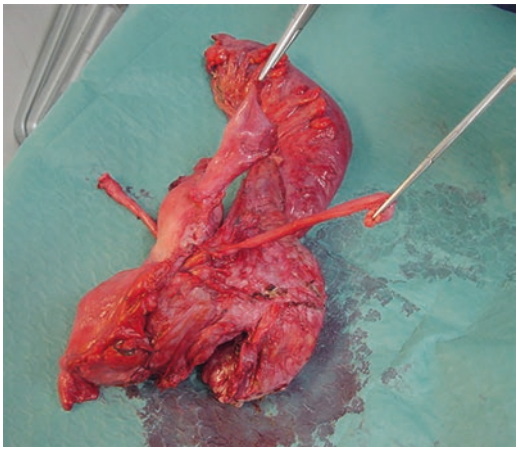

Fig. 10.7 Total supralevator pelvic exenteration – surgical specimen [Northern Gynaecological Oncology Centre (NGOC)]

Pelvic floor reconstruction should be considered in patients treated with pelvic exenteration, as it reduces postoperative morbidity by filling the created cavity during procedure [92]. Especially in a type III pelvic exenteration, a musculocutaneous flap could be used in order to fill the pelvic cavity and allow closure of the perineal defect or to perform a vaginal reconstruction if necessary [92, 100]. In this way, pelvic floor reconstruction decreases significantly the risk of gastrointestinal fistulas and bowel obstruction [92, 100, 115]. Synthetic meshes may be associated with fistulas as well as small bowel and anastomotic leaks [100]. Porcine mesh could be considered as an alternative approach [116].

The wound healing process and the risk of postoperative infection in the surgical field could essentially be improved by transpositioning the omentum, especially in patients with previous radiotherapy [92, 94, 95, 101]. For all these reasons, any pelvic exenterative procedure should be performed in oncology centers with well-documented experience for this type of operation [3, 102].

Complications

Pelvic exenteration is a very extensive surgical procedure that may be associated with significant perioperative mortality and morbidity [3, 92, 103, 117]. In the past, the perioperative mortality rate was essentially high reaching almost 20% [93]. However, improvements in patient selection process and recent advances in surgical technique and perioperative care have reduced significantly the risk of perioperative death [93, 98, 99, 101]. According to recent studies, the perioperative mortality rate in these patients is less than 5% (range 2–14%) [3, 92, 93, 97, 117].

The average perioperative morbidity rate is about 44% (range 33–83%) [93, 96–98]. The average rate of early postoperative complications is approximately 16% (range 16–71%) and mainly affected by tissue damage because of previous radiotherapy and length of the operation [92, 98]. Similarly, the average rate of late postoperative complications is almost 36% (range 36–61%) and mainly influenced by postoperative adhesions, urinary tract infections, and disease recurrence [92, 98].

The most common early postoperative complications in patients who underwent pelvic exenteration are abdominal or pelvic wound infection, abdominal or pelvic wound dehiscence, deep venous thrombosis, pulmonary embolism, bowel obstruction, bowel anastomotic leakage, pelvic abscess, sepsis, entero-cutaneous fistula, entero-vaginal fistula, ureteral leakage, renal failure, lymphatic cyst, and peripheral nerve injury [92, 97–101].

Likewise, the most common late postoperative complications are bowel obstruction, entero-cutaneous fistula, entero-vaginal fistula, ureteral stricture, ureteral obstruction, urinary tract infections, parastomal hernia, and pouch incontinence [92, 97–101].

It is interesting to note that urinary diversion increases the risk of urinary tract infection, sepsis, and renal failure [93]. In contrast, pelvic floor reconstruction decreases significantly the risk of bowel obstruction, pelvic abscess, and fistula formation [92, 115].

Moreover, patients tend to have more psychological, sexual, and social problems and an impaired body image as a result of the exenterative procedure [97]. This is the main reason why rehabilitation programs are absolutely essential for their support [3, 100, 118].

Oncologic Outcome

The overall and disease-free survival rate at 5 years in CC patients treated with pelvic exenteration is about 60% (range 20–73%) and 49%, respectively, when performed with curative intent [3, 92, 93, 96–100, 117]. The median overall and disease-free survival in CC patients after pelvic exenteration is approximately 29.6 months (range 23.0–43.4 months) and 13.4 months (range 1.4–114 months), respectively [93, 101].

The existence of lymph node metastases is associated with worsening prognosis and reduces significantly the overall survival [92, 93, 119]. In CC patients with negative lymph nodes who underwent pelvic exenteration, the median overall survival is about 73.2 months [93]. In contrast, in CC patients with lymph node metastasis who treated with an exenterative procedure, the median overall survival is almost 17.8 months [93].

The status of surgical resection margins in pelvic exenteration represents an independent prognostic factor that essentially affects overall survival [92, 93, 97, 99, 101, 120]. In CC patients with negative resection margins, the overall survival rate at 2 years is about 55.2% [97]. In contrast, in CC patients with positive resection margins, the overall survival rate at 2 years is only 10.2% [97]. Type III pelvic exenteration could possibly increase overall survival by obtaining clear resection margins [92].

In addition, the size of the tumor recurrence has a direct relationship with oncologic outcome [98]. Patients with recurrent disease more than 5 cm have a very poor prognosis, despite pelvic exenteration with complete tumor resection and clear surgical margins [92, 97, 120].

Also, the recurrence-free interval from initial treatment has a direct effect on overall survival [92, 97, 99, 101]. In CC patients with recurrence within 2 years

from initial treatment, the overall survival rate at 5 years is only 16.8% [92, 97]. In CC patients with recurrence between 2 and 5 years from initial treatment, the overall survival rate at 5 years is almost 28% [92, 97]. However, in CC patients with recurrence in more than 5 years from initial treatment, the overall survival rate at 5 years is approximately 83.2% [92, 97].

The median time to recurrence in CC patients with previous pelvic exenteration is almost 6.1 months (range 0.7–47.8 months) [92, 98, 121]. The disease most commonly recurs locally in the pelvis and perineum in 35–60% of patients, while distal metastasis involves the lungs, lymph nodes, and bones in 20–40% of patients [92, 98, 100].

Currently, there are insufficient data to support the role of intraoperative radiotherapy as well as postoperative adjuvant chemotherapy, in order to improve survival and overall outcome after pelvic exenteration, especially in patients with involved resection margins [92, 96, 97]. In addition, there is little data proposing neoadjuvant chemotherapy prior to the exenterative procedure.

Laterally Extended Endopelvic Resection (LEER)

The procedure of laterally extended endopelvic resection (LEER) was initially described by Michael Höckel in 1999 and is based on the ontogenetic compartment theory of locoregional tumor spread [122–125]. It is characterized by the en bloc resection of viscera, sidewall muscles, and major vessels in the lesser pelvis [122–124]. In this way, pelvic exenteration could be laterally extended [123].

Patient Selection

The LEER operation is mainly indicated for patients with locally recurrent CC and pelvic sidewall involvement, who have already treated with pelvic radiotherapy [123, 126, 127]. The procedure could also be used in patients with locally advanced CC and pelvic sidewall involvement as well as in patients with postoperative recurrences without any alternative radiotherapeutic option [123, 126, 127]. Moreover, LEER could possibly be considered in selected CC patients with local recurrence to the pelvic sidewall, even without having any previous radiotherapy [123].

Preoperatively, all patients should have examination under anesthesia and investigation with abdomen and pelvic MRI and whole-body PET-CT [128]. Moreover, a histologic confirmation of disease recurrence should also be available [128].

The eligible patients should have tumor size less than 5 cm, and the disease should be completely excisable and be free from the external iliac vessels [123]. Patient performance status should be compatible with a radical operation [123, 127]. Furthermore, there should not be any available alternative and equally effective treatment option [123].

LEER procedure is contraindicated for patients with distant metastases as well as for patients with multifocal disease [122, 123, 126–128]. Moreover, CC patients with tumor size more than 5 cm and recurrence-free interval less than 5 months from any previous pelvic radiotherapy are not considered suitable for a LEER

operation [122, 123, 126, 128]. Additionally, CC patients with parietal involvement of the sciatic foramen or poor performance status (elderly, significant comorbidities or mental illness) should be excluded from the selection process [122, 123, 126–128].

Technique Description

In the LEER operation, surgical resection planes have been adjusted according to the borders of three pelvic visceral compartments (Müllerian, lower urinary tract, and rectal), in order to excise them en bloc [122, 126, 128, 129]. Lateral resection planes are defined by the acetabulum, obturator membrane, sacrospinous ligament, sacral plexus, and piriformis muscle [122].

The complete specimen in a LEER procedure consists of the urethra, bladder, vagina, uterus, adnexa, and rectum excised en block with internal iliac vessel system and endopelvic part of obturator internus, coccygeus, iliococcygeus, and pubococcygeus muscles of the affected pelvic sidewall (Figs. 10.8, 10.9, and 10.10) [122–124, 126, 127, 129]. Therapeutic pelvic and para-aortic lymph node dissection represents an integral part of LEER operation and should always be performed [123, 126, 127, 129]. However, any gross tumor disruption should be avoided [122].

The provided specimen should be examined with multiple frozen sections, in order to clarify margin status [122, 126, 127]. If margins are clear, then the excisional part of LEER is complete [122]. If there is microscopic residual tumor to the lateral resection margins, then postoperative radiotherapy may be considered [122, 130].

The wound healing process and the risk of postoperative infection in the pelvic surgical field may be improved by transpositioning an omentum majus flap, especially in patients with previous radiotherapy [122, 124, 126, 127, 129]. Moreover, urinary and fecal diversion as well as pelvic floor reconstruction should be performed as in pelvic exenteration.

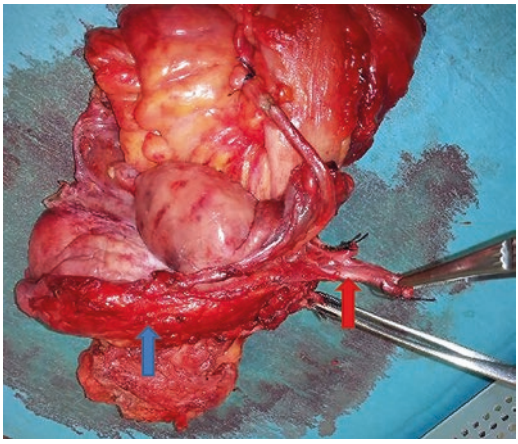

Fig. 10.8 LEER operation – surgical specimen [Northern Gynaecological Oncology Centre (NGOC)]. Blue arrow demonstrates pelvic sidewall muscles, while red arrow demonstrates internal iliac vessels

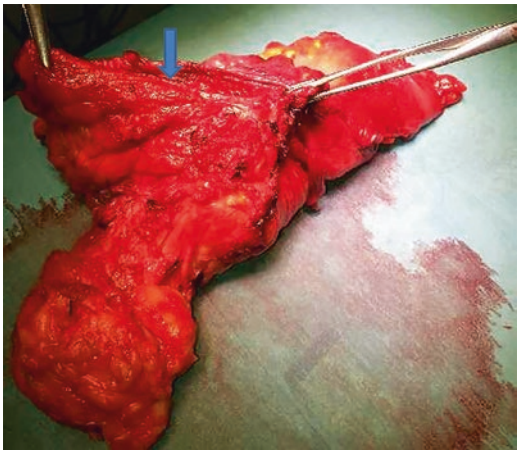

Fig. 10.9 LEER operation – surgical specimen [Northern Gynaecological Oncology Centre (NGOC)]. Blue arrow demonstrates pelvic sidewall muscles

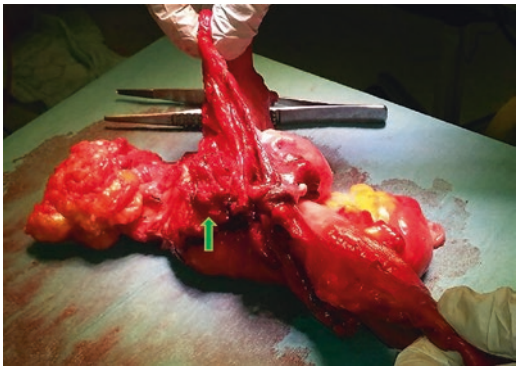

Fig. 10.10 LEER operation – surgical specimen [Northern Gynaecological Oncology Centre (NGOC)]. Green arrow demonstrates obturator muscle

Complications

The average rate of moderate and severe postoperative complications in CC patients treated with LEER operation is essentially high, reaching almost 45% and 9%, respectively [123, 127]. Moreover, the median hospitalization is approximately 25 days (range 14–67 days) [123, 127, 128].

The most common early postoperative complications in patients who underwent LEER operation are abdominal or pelvic wound dehiscence, deep venous thrombosis, pulmonary embolism, bowel obstruction, bowel leakage, pelvic abscess, sepsis, entero-cutaneous fistula, renal failure, lymphatic cyst, and peripheral nerve injury [123, 124, 127, 128]. Similarly, the most common late postoperative complications are parastomal hernia and pouch incontinence [123, 127, 128].

It is worth noting that the most complications are associated with urinary and fecal diversion and pelvic floor reconstruction [127, 128]. The vast majority of severe early complications are caused from failures in urinary diversion, while most late complications are related with urinary and pelvic floor reconstruction [127].

Oncologic Outcome

The main advantage of LEER operation is the provision of locoregional tumor control and long-term survival with a good quality of life in CC patients with locally

advanced or recurrent disease and pelvic sidewall involvement, even if they have already received pelvic radiotherapy [122, 123, 128]. Despite the small number of cases, the overall and disease-free survival rate at 5 years in CC patients treated with LEER is about 44% and 41%, respectively [123]. This is of great importance, because these patients traditionally were not considered eligible for any other salvage treatment [123, 124, 126, 127].

As mentioned previously, margin status is critical, and in addition the presence of lymph node metastasis is associated with a worse prognosis and reduces significantly the overall survival [127]. In CC patients with negative lymph nodes treated with LEER, the overall survival rate at 5 years is approximately 62% [127]. In contrast, CC patients with positive pelvic or para-aortic lymph nodes have overall survival rate at 5 years of only 24% [127]. Nevertheless, pelvic and para-aortic lymph node metastasis shouldn't be considered as an absolute contraindication for a LEER procedure [127].

The LEER operation leads to clear resection margins, and this is the surgical prerequisite in order to achieve locoregional disease control by combining surgery with radiotherapy [122, 130, 131]. Currently, there are insufficient data to support the role of postoperative adjuvant chemotherapy and radiotherapy, in order to improve oncologic outcome after LEER operation [123, 127, 128].

Future Considerations

There is a widespread view that the extended HPV vaccination will significantly decrease the worldwide annual incidence of CC in the near future. In addition, the CC screening program should be carefully designed in order to identify more patients in a preinvasive state as well as at an early stage, thereby increasing the proportion of cases requiring gynecologic surgery, by reducing advanced-stage disease.

Currently, there is a great need for sub-specialization and expertise in the field of gynecological oncology surgery. This is mainly because of the new developments in cancer diagnosis and treatment, as well as all the increasing number of patients diagnosed at an early-stage disease.

Moreover, a detailed pathology report is of great importance in every MDT meeting in order to make the appropriate treatment plan. The pathology report should have all necessary details, including FIGO stage, tumor size and extension, histologic type, depth of cervical stroma invasion, lymphovascular space status, lymph node status, and distant metastases.

Conclusion

Overall, surgical treatment plays a crucial role in the management of CC patients. However, the type and extent of surgical operation as well as the use of alternative treatment options should be carefully individualized according to disease stage, histologic subtype, fertility issues, and performance status (see Fig. 10.2) [3, 6, 7].

References

1. WHO. Estimated cancer incidence, mortality and prevalence worldwide in 2012. GLOBOCAN 2012.
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86.
3. NCCN. Clinical practice guidelines in oncology: cervical cancer. NCCN.org. 2017:1–83.
4. ESGO. Algorithms for management of cervical cancer. ESGO.org. 2010:1–8.
5. Marth C, Landoni F, Mahner S, McCormack M, Gonzalez-Martin A, Colombo N. Cervical cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2017;28(Suppl 4):iv72–83.
6. Chuang L, Temin S, Berek J. Management and care of women with invasive cervical cancer: American Society of Clinical Oncology Resource-Stratified Clinical Practice Guideline Summary. J Oncol Pract. 2016;12(7):693–6.
7. Androutsopoulos G, Kotsopoulos I, Michail G, Decavalas G. Fertility sparing approach in young patients with early stage cervical cancer. Obstet Gynecol Int J. 2017;6(2):00197.
8. Del Priore G. Cone biopsy. An atlas of gynecologic oncology: CRC Press; 2011. p. 68–70.
9. Naik R, Cross P, Nayar A, Mayadevi S, Lopes A, Godfrey K, et al. Conservative surgical management of small-volume stage IB1 cervical cancer. BJOG. 2007;114(8):958–63.
10. Biliatis I, Kucukmetin A, Patel A, Ratnavelu N, Cross P, Chattopadhyay S, et al. Small volume stage 1B1 cervical cancer: is radical surgery still necessary? Gynecol Oncol. 2012;126(1):73–7.
11. Androutsopoulos G, Kotsopoulos I, Korompelis P, Decavalas G. Does conservative surgical management of early stage cervical cancer represent a persistent dilemma in young patients? OA J Surg. 2017;3(3):555611.
12. Viswanathan A, Deavers M, Jhingran A, Ramirez P, Levenback C, Eifel P. Small cell neuroendocrine carcinoma of the cervix: outcome and patterns of recurrence. Gynecol Oncol. 2004;93(1):27–33.
13. Young R, Clement P. Endocervical adenocarcinoma and its variants: their morphology and differential diagnosis. Histopathology. 2002;41(3):185–207.
14. Lopes T, Spirtos N, Naik R, Monaghan J. Operations on the cervix. Bonney's gynaecological surgery. Chichester: Wiley-Blackwell; 2010. p. 81–98.
15. Miroshnichenko G, Parva M, Holtz D, Klemens J, Dunton C. Interpretability of excisional biopsies of the cervix: cone biopsy and loop excision. J Low Genit Tract Dis. 2009;13(1):10–2.
16. Fanning J, Padratzik J. Cold knife conization vs. LEEP. Are they the same procedure? J Reprod Med. 2002;47(1):33–5.
17. Kim M, Kim M, Kim J, Chung H, Park N, Song Y, et al. Loop electrosurgical excision procedure findings for identification of patients with early-stage cervical cancer suitable for less radical surgery. Int J Gynecol Cancer. 2012;22(7):1214–9.
18. Andikyan V, Khoury-Collado F, Denesopolis J, Park K, Hussein Y, Brown C, et al. Cervical conization and sentinel lymph node mapping in the treatment of stage I cervical cancer: is less enough? Int J Gynecol Cancer. 2014;24(1):113–7.
19. Kyrgiou M, Koliopoulos G, Martin-Hirsch P, Arbyn M, Prendiville W, Paraskevaidis E. Obstetric outcomes after conservative treatment for intraepithelial or early invasive cervical lesions: systematic review and meta-analysis. Lancet. 2006;367(9509):489–98.
20. Wright J, NathavithArana R, Lewin S, Sun X, Deutsch I, Burke W, et al. Fertility-conserving surgery for young women with stage IA1 cervical cancer: safety and access. Obstet Gynecol. 2010;115(3):585–90.
21. Ditto A, Martinelli F, Bogani G, Fischetti M, Di Donato V, Lorusso D, et al. Fertility-sparing surgery in early-stage cervical cancer patients: oncologic and reproductive outcomes. Int J Gynecol Cancer. 2015;25(3):493–7.

22. Haller H, Krasevic M, Mamula O, Brncic-Fischer A, Eminovic S, Manestar M. Treatment and outcome of stage Ia1 squamous cell carcinoma of the uterine cervix. Int J Gynaecol Obstet. 2011;113(1):72–5.
23. Raspagliesi F, Ditto A, Quattrone P, Solima E, Fontanelli R, Dousias V, et al. Prognostic factors in microinvasive cervical squamous cell cancer: long-term results. Int J Gynecol Cancer. 2005;15(1):88–93.
24. Kyrgiou M, Athanasiou A, Paraskevaidi M, Mitra A, Kalliala I, Martin-Hirsch P, et al. Adverse obstetric outcomes after local treatment for cervical preinvasive and early invasive disease according to cone depth: systematic review and meta-analysis. BMJ. 2016;354:i3633.
25. Nam K, Kwon J, Kim Y, Park Y. Pregnancy outcome after cervical conization: risk factors for preterm delivery and the efficacy of prophylactic cerclage. J Gynecol Oncol. 2010;21(4):225–9.
26. Dargent D, Brun J, Roy M, Mathevet P, Remy I. La trache'-lectomie e'largie (TE), une alternative a' l'hyste'rectomie radicale dans le traitement des cancers infiltrants de'veloppe's sur la face externe du col ute'rin. JOBGYN. 1994;2:285–92.
27. Dargent D, Martin X, Sacchetoni A, Mathevet P. Laparoscopic vaginal radical trachelectomy: a treatment to preserve the fertility of cervical carcinoma patients. Cancer. 2000;88(8):1877–82.
28. Smith J, Boyle D, Corless D, Ungar L, Lawson A, Del Priore G, et al. Abdominal radical trachelectomy: a new surgical technique for the conservative management of cervical carcinoma. Br J Obstet Gynaecol. 1997;104(10):1196–200.
29. Schneider A, Erdemoglu E, Chiantera V, Reed N, Morice P, Rodolakis A, et al. Clinical recommendation radical trachelectomy for fertility preservation in patients with early-stage cervical cancer. Int J Gynecol Cancer. 2012;22(4):659–66.
30. Abu-Rustum N, Sonoda Y, Black D, Levine D, Chi D, Barakat R. Fertility-sparing radical abdominal trachelectomy for cervical carcinoma: technique and review of the literature. Gynecol Oncol. 2006;103(3):807–13.
31. Peppercorn P, Jeyarajah A, Woolas R, Shepherd J, Oram D, Jacobs I, et al. Role of MR imaging in the selection of patients with early cervical carcinoma for fertility-preserving surgery: initial experience. Radiology. 1999;212(2):395–9.
32. Balleyguier C, Sala E, Da Cunha T, Bergman A, Brkljacic B, Danza F, et al. Staging of uterine cervical cancer with MRI: guidelines of the European Society of Urogenital Radiology. Eur Radiol. 2011;21(5):1102–10.
33. Lakhman Y, Akin O, Park K, Sarasohn D, Zheng J, Goldman D, et al. Stage IB1 cervical cancer: role of preoperative MR imaging in selection of patients for fertility-sparing radical trachelectomy. Radiology. 2013;269(1):149–58.
34. Diaz JP, Sonoda Y, Leitao M, Zivanovic O, Brown C, Chi D, et al. Oncologic outcome of fertility-sparing radical trachelectomy versus radical hysterectomy for stage IB1 cervical carcinoma. Gynecol Oncol. 2008;111(2):255–60.
35. Cao D, Yang J, Wu X, Chen Y, Li L, Liu K, et al. Comparisons of vaginal and abdominal radical trachelectomy for early-stage cervical cancer: preliminary results of a multi-center research in China. Br J Cancer. 2013;109(11):2778–82.
36. Chan K, Naik R. Advances in surgical treatment of cervical cancer. Womens Health (Lond). 2008;4(3):245–56.
37. Ramirez P, Pareja R, Rendon G, Millan C, Frumovitz M, Schmeler K. Management of low-risk early-stage cervical cancer: should conization, simple trachelectomy, or simple hysterectomy replace radical surgery as the new standard of care? Gynecol Oncol. 2014;132(1):254–9.
38. Bentivegna E, Gouy S, Maulard A, Chargari C, Leary A, Morice P. Oncological outcomes after fertility-sparing surgery for cervical cancer: a systematic review. Lancet Oncol. 2016;17(6):e240–53.
39. Hauerberg L, Hogdall C, Loft A, Ottosen C, Bjoern S, Mosgaard B, et al. Vaginal radical trachelectomy for early stage cervical cancer. Results of the Danish National Single Center Strategy. Gynecol Oncol. 2015;138(2):304–10.

40. Ungar L, Palfalvi L, Hogg R, Siklos P, Boyle D, Del Priore G, et al. Abdominal radical trachelectomy: a fertility-preserving option for women with early cervical cancer. BJOG. 2005;112(3):366–9.
41. Lopes T, Spirtos N, Naik R, Monaghan J. Cervical cancer. Bonney's gynaecological surgery. Chichester: Wiley-Blackwell; 2010. p. 192–215.
42. Plante M, Gregoire J, Renaud M, Roy M. The vaginal radical trachelectomy: an update of a series of 125 cases and 106 pregnancies. Gynecol Oncol. 2011;121(2):290–7.
43. Einstein M, Park K, Sonoda Y, Carter J, Chi D, Barakat R, et al. Radical vaginal versus abdominal trachelectomy for stage IB1 cervical cancer: a comparison of surgical and pathologic outcomes. Gynecol Oncol. 2009;112(1):73–7.
44. Kucukmetin A, Biliatis I, Ratnavelu N, Patel A, Cameron I, Ralte A, et al. Laparoscopic radical trachelectomy is an alternative to laparotomy with improved perioperative outcomes in patients with early-stage cervical cancer. Int J Gynecol Cancer. 2014;24(1):135–40.
45. Vieira M, Rendon G, Munsell M, Echeverri L, Frumovitz M, Schmeler K, et al. Radical trachelectomy in early-stage cervical cancer: a comparison of laparotomy and minimally invasive surgery. Gynecol Oncol. 2015;138(3):585–9.
46. Johansen G, Lonnerfors C, Falconer H, Persson J. Reproductive and oncologic outcome following robot-assisted laparoscopic radical trachelectomy for early stage cervical cancer. Gynecol Oncol. 2016;141(1):160–5.
47. Cibula D, Ungar L, Palfalvi L, Bino B, Kuzel D. Laparoscopic abdominal radical trachelectomy. Gynecol Oncol. 2005;97(2):707–9.
48. Ungar L, Palfalvi L, Boyle D, Del Priore G, Smith RJ. Radical abdominal trachelectomy. In: An atlas of gynecologic oncology: CRC Press; 2011. p. 97–104.
49. Wethington S, Sonoda Y, Park K, Alektiar K, Tew W, Chi DS, et al. Expanding the indications for radical trachelectomy: a report on 29 patients with stage IB1 tumors measuring 2 to 4 centimeters. Int J Gynecol Cancer. 2013;23(6):1092–8.
50. Plante M, Roy M. Radical vaginal trachelectomy. In: An atlas of gynecologic oncology: CRC Press; 2011. p. 88–96.
51. Lanowska M, Mangler M, Spek A, Grittner U, Hasenbein K, Chiantera V, et al. Radical vaginal trachelectomy (RVT) combined with laparoscopic lymphadenectomy: prospective study of 225 patients with early-stage cervical cancer. Int J Gynecol Cancer. 2011;21(8):1458–64.
52. Alexander-Sefre F, Chee N, Spencer C, Menon U, Shepherd J. Surgical morbidity associated with radical trachelectomy and radical hysterectomy. Gynecol Oncol. 2006;101(3):450–4.
53. Pareja R, Rendon G, Sanz-Lomana C, Monzon O, Ramirez P. Surgical, oncological, and obstetrical outcomes after abdominal radical trachelectomy – a systematic literature review. Gynecol Oncol. 2013;131(1):77–82.
54. Gien L, Covens A. Fertility-sparing options for early stage cervical cancer. Gynecol Oncol. 2010;117(2):350–7.
55. Okugawa K, Kobayashi H, Sonoda K, Kaneki E, Kawano Y, Hidaka N, et al. Oncologic and obstetric outcomes and complications during pregnancy after fertility-sparing abdominal trachelectomy for cervical cancer: a retrospective review. Int J Clin Oncol. 2017;22(2):340–6.
56. Lintner B, Saso S, Tarnai L, Novak Z, Palfalvi L, Del Priore G, et al. Use of abdominal radical trachelectomy to treat cervical cancer greater than 2 cm in diameter. Int J Gynecol Cancer. 2013;23(6):1065–70.
57. Speiser D, Mangler M, Kohler C, Hasenbein K, Hertel H, Chiantera V, et al. Fertility outcome after radical vaginal trachelectomy: a prospective study of 212 patients. Int J Gynecol Cancer. 2011;21(9):1635–9.
58. Kasuga Y, Nishio H, Miyakoshi K, Sato S, Sugiyama J, Matsumoto T, et al. Pregnancy outcomes after abdominal radical Trachelectomy for early-stage cervical Cancer: a 13-year experience in a single tertiary-care center. Int J Gynecol Cancer. 2016;26(1):163–8.
59. Boss E, van Golde R, Beerendonk C, Massuger L. Pregnancy after radical trachelectomy: a real option? Gynecol Oncol. 2005;99(3 Suppl 1):S152–6.
60. Wertheim E. Zur frag der radikaloperation beim uteruskrebs. Arch Gynakol. 1900;61:627.

61. Dargent D, Mathevet P. Schauta's vaginal hysterectomy combined with laparoscopic lymphadenectomy. Baillieres Clin Obstet Gynaecol. 1995;9(4):691–705.
62. Boyle D. Radical abdominal hysterectomy. An atlas of gynecologic oncology: CRC Press, 2011:71–77.
63. Maneo A, Landoni F, Cormio G, Colombo A, Mangioni C. Radical hysterectomy for recurrent or persistent cervical cancer following radiation therapy. Int J Gynecol Cancer. 1999;9(4):295–301.
64. Landoni F, Maneo A, Colombo A, Placa F, Milani R, Perego P, et al. Randomised study of radical surgery versus radiotherapy for stage Ib-IIa cervical cancer. Lancet. 1997;350(9077):535–40.
65. Keys H, Bundy B, Stehman F, Muderspach L, Chafe W, Suggs C 3rd, et al. Cisplatin, radiation, and adjuvant hysterectomy compared with radiation and adjuvant hysterectomy for bulky stage IB cervical carcinoma. N Engl J Med. 1999;340(15):1154–61.
66. Morris M, Eifel P, Lu J, Grigsby P, Levenback C, Stevens R, et al. Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer. N Engl J Med. 1999;340(15):1137–43.
67. Kokka F, Bryant A, Brockbank E, Powell M, Oram D. Hysterectomy with radiotherapy or chemotherapy or both for women with locally advanced cervical cancer. Cochrane Database Syst Rev. 2015;(4):Cd010260.
68. Bradbury M, Founta C, Taylor W, Kucukmetin A, Naik R, Ang C. Pathological risk factors and outcomes in women with stage IB2 cervical cancer treated with primary radical surgery versus chemoradiotherapy. Int J Gynecol Cancer. 2015;25(8):1476–83.
69. Gaffney D, Erickson-Wittmann B, Jhingran A, Mayr N, Puthawala A, Moore D, et al. ACR appropriateness criteria(R) on advanced cervical Cancer expert panel on radiation oncology-gynecology. Int J Radiat Oncol Biol Phys. 2011;81(3):609–14.
70. Monk B, Tewari K, Koh W. Multimodality therapy for locally advanced cervical carcinoma: state of the art and future directions. J Clin Oncol. 2007;25(20):2952–65.
71. Benedetti-Panici P, Maneschi F, D'Andrea G, Cutillo G, Rabitti C, Congiu M, et al. Early cervical carcinoma: the natural history of lymph node involvement redefined on the basis of thorough parametrectomy and giant section study. Cancer. 2000;88(10):2267–74.
72. ConCerv trial. https://clinicaltrials.gov/ct2/show/study/NCT01048853
73. SHAPE trial. https://clinicaltrials.gov/show/NCT01658930
74. Landoni F, Maneo A, Cormio G, Perego P, Milani R, Caruso O, et al. Class II versus class III radical hysterectomy in stage IB-IIA cervical cancer: a prospective randomized study. Gynecol Oncol. 2001;80(1):3–12.
75. Marnitz S, Kohler C, Affonso R, Schneider A, Chiantera V, Tsounoda A, et al. Validity of laparoscopic staging to avoid adjuvant chemoradiation following radical surgery in patients with early cervical cancer. Oncology. 2012;83(6):346–53.
76. Kucukmetin A, Biliatis I, Naik R, Bryant A. Laparoscopically assisted radical vaginal hysterectomy versus radical abdominal hysterectomy for the treatment of early cervical cancer. Cochrane Database Syst Rev. 2013;(10):Cd006651.
77. Nam J, Park J, Kim D, Kim J, Kim Y, Kim Y. Laparoscopic versus open radical hysterectomy in early-stage cervical cancer: long-term survival outcomes in a matched cohort study. Ann Oncol. 2012;23(4):903–11.
78. Wang Y, Deng L, Xu H, Zhang Y, Liang Z. Laparoscopy versus laparotomy for the management of early stage cervical cancer. BMC Cancer. 2015;15:928.
79. Jackson K, Das N, Naik R, Lopes A, Godfrey K, Hatem M, et al. Laparoscopically assisted radical vaginal hysterectomy vs. radical abdominal hysterectomy for cervical cancer: a match controlled study. Gynecol Oncol. 2004;95(3):655–61.
80. Querleu D, Morrow C. Classification of radical hysterectomy. Lancet Oncol. 2008;9(3):297–303.
81. Cibula D, Abu-Rustum N, Benedetti-Panici P, Kohler C, Raspagliesi F, Querleu D, et al. New classification system of radical hysterectomy: emphasis on a three-dimensional anatomic template for parametrial resection. Gynecol Oncol. 2011;122(2):264–8.

82. Cormier B, Diaz J, Shih K, Sampson R, Sonoda Y, Park K, et al. Establishing a sentinel lymph node mapping algorithm for the treatment of early cervical cancer. Gynecol Oncol. 2011;122(2):275–80.
83. Lecuru F, Mathevet P, Querleu D, Leblanc E, Morice P, Darai E, et al. Bilateral negative sentinel nodes accurately predict absence of lymph node metastasis in early cervical cancer: results of the SENTICOL study. J Clin Oncol. 2011;29(13):1686–91.
84. Cibula D, Abu-Rustum N, Dusek L, Slama J, Zikan M, Zaal A, et al. Bilateral ultrastaging of sentinel lymph node in cervical cancer: lowering the false-negative rate and improving the detection of micrometastasis. Gynecol Oncol. 2012;127(3):462–6.
85. Cibula D, Velechovska P, Slama J, Fischerova D, Pinkavova I, Pavlista D, et al. Late morbidity following nerve-sparing radical hysterectomy. Gynecol Oncol. 2010;116(3):506–11.
86. Ditto A, Martinelli F, Hanozet F, Reato C, Solima E, Zanaboni F, et al. Class III NSRH: oncological outcome in 170 cervical cancer patients. Gynecol Oncol. 2010;119(2):192–7.
87. Sedlis A, Bundy B, Rotman M, Lentz S, Muderspach L, Zaino R. A randomized trial of pelvic radiation therapy versus no further therapy in selected patients with stage IB carcinoma of the cervix after radical hysterectomy and pelvic lymphadenectomy: a Gynecologic Oncology Group study. Gynecol Oncol. 1999;73(2):177–83.
88. Monk B, Wang J, Im S, Stock R, Peters W 3rd, Liu P, et al. Rethinking the use of radiation and chemotherapy after radical hysterectomy: a clinical-pathologic analysis of a Gynecologic Oncology Group/Southwest Oncology Group/Radiation Therapy Oncology Group trial. Gynecol Oncol. 2005;96(3):721–8.
89. Rotman M, Sedlis A, Piedmonte M, Bundy B, Lentz S, Muderspach L, et al. A phase III randomized trial of postoperative pelvic irradiation in stage IB cervical carcinoma with poor prognostic features: follow-up of a gynecologic oncology group study. Int J Radiat Oncol Biol Phys. 2006;65(1):169–76.
90. Peters W 3rd, Liu P, Barrett R 2nd, Stock R, Monk B, Berek J, et al. Concurrent chemotherapy and pelvic radiation therapy compared with pelvic radiation therapy alone as adjuvant therapy after radical surgery in high-risk early-stage cancer of the cervix. J Clin Oncol. 2000;18(8):1606–13.
91. Brunschwig A. Complete excision of pelvic viscera for advanced carcinoma; a one-stage abdominoperineal operation with end colostomy and bilateral ureteral implantation into the colon above the colostomy. Cancer. 1948;1(2):177–83.
92. Sardain H, Lavoue V, Redpath M, Bertheuil N, Foucher F, Leveque J. Curative pelvic exenteration for recurrent cervical carcinoma in the era of concurrent chemotherapy and radiation therapy. A systematic review. Eur J Surg Oncol. 2015;41(8):975–85.
93. Graves S, Seagle B, Strohl A, Shahabi S, Nieves-Neira W. Survival after pelvic exenteration for cervical cancer: a National Cancer Database study. Int J Gynecol Cancer. 2017;27(2):390–5.
94. Lopes T, Spirtos N, Naik R, Monaghan J. Exenterative surgery. In: Bonney's gynaecological surgery: Wiley-Blackwell; 2010. p. 222–30.
95. Monaghan J. Central recurrent cervical cancer. In: An atlas of gynecologic oncology: CRC Press; 2011. p. 105–11.
96. Marnitz S, Dowdy S, Lanowska M, Schneider A, Podratz K, Kohler C. Exenterations 60 years after first description: results of a survey among US and German gynecologic oncology centers. Int J Gynecol Cancer. 2009;19(5):974–7.
97. Marnitz S, Kohler C, Muller M, Behrens K, Hasenbein K, Schneider A. Indications for primary and secondary exenterations in patients with cervical cancer. Gynecol Oncol. 2006;103(3):1023–30.
98. Yoo H, Lim M, Seo S, Kang S, Yoo C, Kim J, et al. Pelvic exenteration for recurrent cervical cancer: ten-year experience at National Cancer Center in Korea. J Gynecol Oncol. 2012;23(4):242–50.
99. Berek J, Howe C, Lagasse L, Hacker N. Pelvic exenteration for recurrent gynecologic malignancy: survival and morbidity analysis of the 45-year experience at UCLA. Gynecol Oncol. 2005;99(1):153–9.

100. Goldberg G, Sukumvanich P, Einstein M, Smith H, Anderson P, Fields A. Total pelvic exenteration: the Albert Einstein College of Medicine/Montefiore Medical Center Experience (1987 to 2003). Gynecol Oncol. 2006;101(2):261–8.
101. Chiantera V, Rossi M, De Iaco P, Koehler C, Marnitz S, Fagotti A, et al. Morbidity after pelvic exenteration for gynecological malignancies: a retrospective multicentric study of 230 patients. Int J Gynecol Cancer. 2014;24(1):156–64.
102. Ungar L, Palfalvi L, Novak Z. Primary pelvic exenteration in cervical cancer patients. Gynecol Oncol. 2008;111(2 Suppl):S9–12.
103. Ang C, Bryant A, Barton D, Pomel C, Naik R. Exenterative surgery for recurrent gynaecological malignancies. Cochrane Database Syst Rev 2014(2):Cd010449.
104. Guimaraes G, Baiocchi G, Ferreira F, Kumagai L, Fallopa C, Aguiar S, et al. Palliative pelvic exenteration for patients with gynecological malignancies. Arch Gynecol Obstet. 2011;283(5):1107–12.
105. Magrina J, Stanhope C, Weaver A. Pelvic exenterations: supralevator, infralevator, and with vulvectomy. Gynecol Oncol. 1997;64(1):130–5.
106. Katory M, McLean R, Paez E, Kucukmetin A, Naik R. Short- and long-term outcomes following pelvic exenteration for gynae-oncological and colorectal cancers: a 9 year consecutive single-centre cohort study. Int J Surg. 2017;43:38–45.
107. Witjes A, Lebret T, Comperat E, Cowan N, De Santis M, Bruins H, et al. Updated 2016 EAU guidelines on muscle-invasive and metastatic bladder cancer. Eur Urol. 2017;71(3):462–75.
108. Lee R, Abol-Enein H, Artibani W, Bochner B, Dalbagni G, Daneshmand S, et al. Urinary diversion after radical cystectomy for bladder cancer: options, patient selection, and outcomes. BJU Int. 2014;113(1):11–23.
109. Shah N, Ward K, Plaxe S, Saenz C, McHale M. Urinary diversions: a time to enrich surgical training? Gynecol Oncol. 2016;140(1):120–3.
110. Colombo R, Naspro R. Ileal conduit as the standard for urinary diversion after radical cystectomy for bladder cancer. Eur Urol Suppl. 2010;9(10):736–44.
111. Davis N, Burke J, McDermott T, Flynn R, Manecksha R, Thornhill J. Bricker versus Wallace anastomosis: a meta-analysis of ureteroenteric stricture rates after ileal conduit urinary diversion. Can Urol Assoc J. 2015;9(5–6):E284–90.
112. Bricker E. Bladder substitution after pelvic evisceration. Surg Clin North Am. 1950;30(5):1511–21.
113. Wallace D. Ureteric diversion using a conduit: a simplified technique. Br J Urol. 1966;38(5):522–7.
114. Wallace D. Uretero-ileostomy. Br J Urol. 1970;42(5):529–34.
115. Miller B, Morris M, Gershenson D, Levenback C, Burke T. Intestinal fistulae formation following pelvic exenteration: a review of the University of Texas M. D. Anderson Cancer Center experience, 1957–1990. Gynecol Oncol. 1995;56(2):207–10.
116. Musters G, Bemelman W, Bosker R, Burger J, van Duijvendijk P, van Etten B, et al. Randomized controlled multicentre study comparing biological mesh closure of the pelvic floor with primary perineal wound closure after extralevator abdominoperineal resection for rectal cancer (BIOPEX-study). BMC Surg. 2014;14:58.
117. Morley G, Hopkins M, Lindenauer S, Roberts J. Pelvic exenteration, University of Michigan: 100 patients at 5 years. Obstet Gynecol. 1989;74(6):934–43.
118. Turns D. Psychosocial issues: pelvic exenterative surgery. J Surg Oncol. 2001;76(3):224–36.
119. Wang C, Lai C, Huang H, Hong J, Chou H, Huang K, et al. Recurrent cervical carcinoma after primary radical surgery. Am J Obstet Gynecol. 1999;181(3):518–24.
120. Shingleton H, Soong S, Gelder M, Hatch K, Baker V, Austin J Jr. Clinical and histopathologic factors predicting recurrence and survival after pelvic exenteration for cancer of the cervix. Obstet Gynecol. 1989;73(6):1027–34.
121. Benn T, Brooks R, Zhang Q, Powell M, Thaker P, Mutch D, et al. Pelvic exenteration in gynecologic oncology: a single institution study over 20 years. Gynecol Oncol. 2011;122(1):14–8.

122. Höckel M. Laterally extended endopelvic resection: surgical treatment of infrailiac pelvic wall recurrences of gynecologic malignancies. Am J Obstet Gynecol. 1999;180(2 Pt 1):306–12.
123. Höckel M. Laterally extended endopelvic resection. Novel surgical treatment of locally recurrent cervical carcinoma involving the pelvic side wall. Gynecol Oncol. 2003;91(2):369–77.
124. Höckel M. Laterally extended endopelvic resection (LEER) – principles and practice. Gynecol Oncol. 2008;111(Suppl 2):S13–7.
125. Höckel M, Hentschel B, Horn L. Association between developmental steps in the organogenesis of the uterine cervix and locoregional progression of cervical cancer: a prospective clinicopathological analysis. Lancet Oncol. 2014;15(4):445–56.
126. Höckel M. Laterally extended endopelvic resection. In: An atlas of gynecologic oncology: CRC Press; 2011. p. 120–6.
127. Höckel M, Wolf B, Hentschel B, Horn L. Surgical treatment and histopathological assessment of advanced cervicovaginal carcinoma: a prospective study and retrospective analysis. Eur J Cancer. 2017;70:99–110.
128. Höckel M, Horn L, Einenkel J. (Laterally) extended endopelvic resection: surgical treatment of locally advanced and recurrent cancer of the uterine cervix and vagina based on ontogenetic anatomy. Gynecol Oncol. 2012;127(2):297–302.
129. Höckel M. Long-term experience with (laterally) extended endopelvic resection (LEER) in relapsed pelvic malignancies. Curr Oncol Rep. 2015;17(3):435.
130. Höckel M, Sclenger K, Hamm H, Knapstein P, Hohenfellner R, Rosler H. Five-year experience with combined operative and radiotherapeutic treatment of recurrent gynecologic tumors infiltrating the pelvic wall. Cancer. 1996;77(9):1918–33.
131. Höckel M, Knapstein P. The combined operative and radiotherapeutic treatment (CORT) of recurrent tumors infiltrating the pelvic wall: first experience with 18 patients. Gynecol Oncol. 1992;46(1):20–8.

Management of Recurrent Uterine Cervical Cancer

11

George Zarkavelis, Alexandra Papadaki, Aristides Kefas, Ioannis Zerdes, Konstantina Tatsi, and Stergios Boussios

Although there are an increasing number of chemotherapy regimens and immunomodulatory factors that have become part of the everyday clinical practice, uterine cervical cancer still remains an orphan disease. Statistics and epidemiology place cervical cancer in the fourth place among most common causes of mortality in women especially in the developing world. The highest mortality rates are observed in less developed countries of the world. Overall, cervical cancer is the fourth most common cancer across the globe in the female gender with 528,000 cases diagnosed annually and 266,000 disease-related deaths [1].

Many efforts have been made the last decades regarding both primary and secondary prevention strategies, namely, due to the fact that most cases are diagnosed in more advanced stages. In addition, therapeutics of early-stage cervical cancer has accomplished a survival rate ranging from 60% to 80%. Pap smear screening and the incorporation of human papillomavirus infection (HPV) DNA testing combined with the slow growth rate of cervical cancer have led to a decline in the number of invasive cervix cancer cases [2].

The most effective prevention strategy is the implement of HPV vaccination which reduces the incidence of the disease. According to current guidelines, optimal effectiveness of vaccination can be achieved when implemented prior to HPV exposure, whereas the vaccine seems to be ineffective when a woman has already been exposed to the virus [3].

For very early stages, surgical methods consisting of either loop electrosurgical excision procedure (LEEP) or conization are acceptable treatment options when fertility sparing is desired (Fig. 11.1). Radical hysterectomies with lymph node

G. Zarkavelis · A. Papadaki · I. Zerdes · S. Boussios (✉)
Department of Medical Oncology, Ioannina University Hospital, Ioannina, Greece

A. Kefas
Department of Medicine, Ioannina University Medical School, Ioannina, Greece

K. Tatsi
Gynaecology Unit, General Hospital "G. Hatzikosta", Ioannina, Greece

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_11

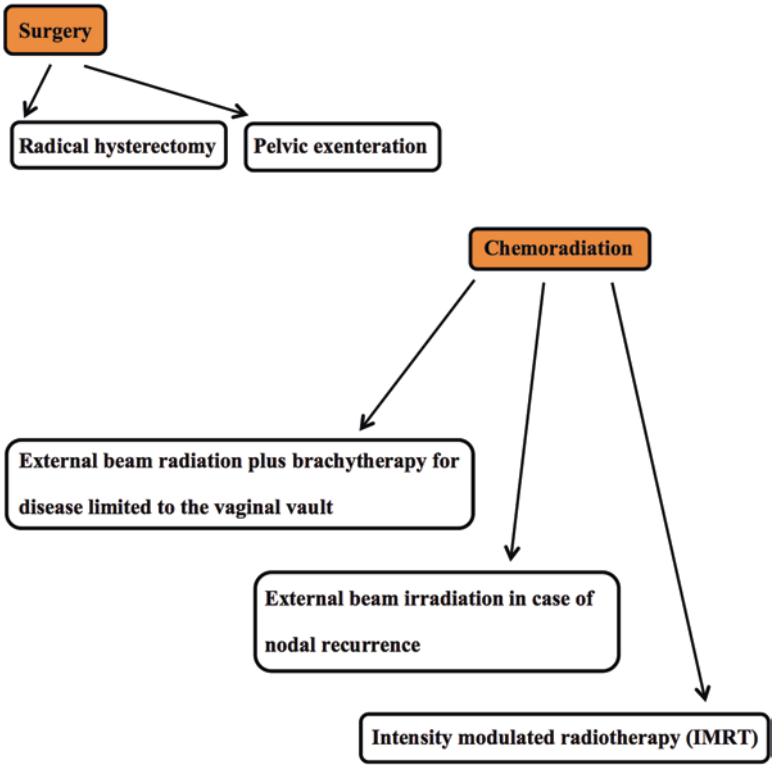

Fig. 11.1 Therapeutic landscape of recurrent cervical cancer

dissection or cisplatin-based chemoradiation are the treatments of choice in more advanced stages. Approximately 15–61% of women will relapse after the completion of primary treatment within 24 months [4]. At that point, both surgical techniques and radiotherapy can serve as palliative or potentially curative modalities. Overall, the extent of the disease in the metastatic setting usually precludes the scenario of curative interventions. Palliative cisplatin-based chemotherapy is preferred for alleviating the symptoms when metastatic or recurrent disease is documented. For this group of patients, the prognosis still remains poor. The 5-year overall survival (OS) of patients with recurrent or metastatic disease is 5% [4].

For women diagnosed with recurrent or metastatic disease not amenable to curative interventions, the primary goal of treatment is to palliate the symptoms and prolong survival with the use of systemic therapy [5]. To date, these patients constitute a high-risk population for whom more research is of great need in order to improve efficacy and overcome the adverse effects of treatment. There is currently no standard of care for second-line treatment which represents a significant but unmet clinical need. The main challenge is the accrual of patients in clinical trials in order to test novel therapies with statistical power.

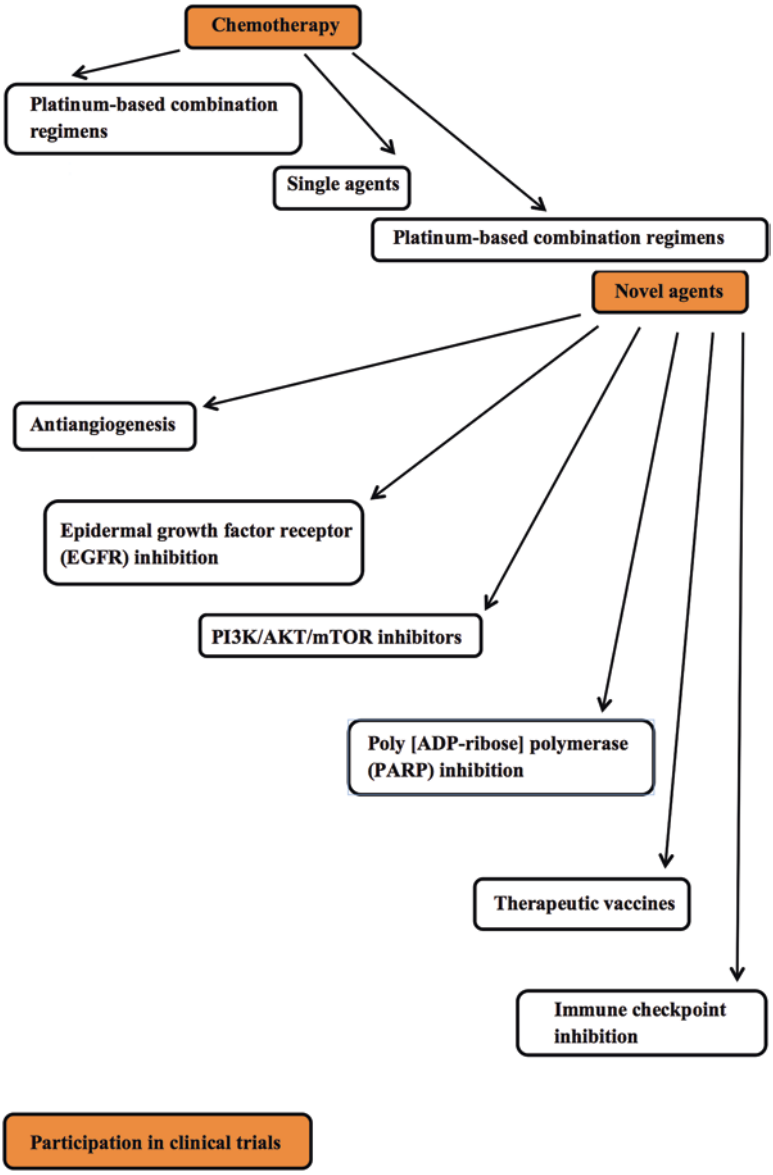

Fig. 11.1 (continued)

Recurrence Pattern

The options for intervention regarding recurrent cervical cancer are strongly associated to the site of recurrence as well as the extent of the disease [6]. More than half of the patients have undergone radiotherapy in the primary setting making it extremely difficult to receive additional radiation to the pelvic area. This group of patients usually fare far worse at the time of relapse due to the narrow spectrum of therapeutic modalities that can be applied. Sites of recurrence are either central pelvic, lateral pelvic, or extra pelvic [7]. When extra pelvic disease is documented, the most common metastatic areas are the para-aortic lymph nodes, liver, lung, and bones. Among 327 women with relapsed cervical cancer, 36.7% had disease located in the central pelvis, 9.5% in the lateral pelvis, 21% in the vaginal vault, and 4.9% in the lymph nodes, and in 4.3% were detected both pelvic and extrapelvic metastases [8].

The relapse rate of cervical cancer ranges between 11% and 22% in the International Federation of Gynecology and Obstetrics (FIGO) IB–IIA stages and between 28% and 64% in IIB–IVA stages, respectively [6]. As the bulk of pelvic tumor increases, the amount of patients with disease recurrence in the pelvis as the main site of failure exceeds that of distant metastatic sites. Perez et al. have reported a pelvic failure rate of 10% in stage IB, 17% in stage IIA, 23% in stage IIB, 42% in stage II disease, and 74% in stage IVA after prior use of radiotherapy [9].

Central or Lateral Pelvis Recurrence in Patients Treated Only with Radical Hysterectomy in the Primary Setting

The treatment of choice for this cohort of patients is concurrent chemoradiation [10]. Cisplatin chemosensitization is established, while caution should be given to the fact that prior surgical adhesions may increase the amount of radiation delivered to the bowel. In addition, former surgery limits the brachytherapy options especially in the vaginal vault. However, disease limited to the vaginal vault can be treated with external beam radiation plus brachytherapy. On the contrary, in case of nodal recurrence, external irradiation is the only modality taking into consideration the systemic profile of the disease. The obstacles can be overcome with the implementation of intensity-modulated radiotherapy (IMRT) which spares the surrounding tissues from excessive radiation while delivering therapeutic doses specifically to the target lesions. Overall, the 5-year survival rates depend on the recurrence site and range from 6% to 77% [11].

Central Pelvis Recurrence in Irradiated Patients

Surgery presents the treatment of choice in this group of patients with a high range of complications accompanying surgical interventions. Fistulas, genitourinary or gastrointestinal injuries, and sepsis are common complications of radical

hysterectomy after prior irradiation and are associated to the extent of tumor relapse. Radical hysterectomy is the treatment of choice for selected patients with low-volume disease. On the contrary, pelvic exenteration seems to be the only possible surgical procedure with therapeutic intent in patients with large-volume disease accompanied by high rates of mortality [12, 13]. This surgical approach includes the formation of terminal colostomy and ileal conduit as urinary diversion [14]. Negative lymph nodes, negative postsurgical margins, low-volume disease, and a former long disease-free interval (DFI) are correlated with higher 5-year survival rates [13]. Positron emission tomography–computed tomography (PET/CT) can provide with significant information regarding the extent of disease prior to surgery [15]. Intraoperative radiation can be used in cases of positive margins on frozen section analysis. Finally, urinary tract infections, pyelonephritis, gastrointestinal fistulas, and surgical failures are serious complications in such extended surgery in a former irradiated area, whereas gastrointestinal fistulas could be avoided with pelvic floor and vaginal reconstruction with the use of myocutaneous flaps [12, 16].

Lateral Pelvis Recurrence in Previously Irradiated Patients

Pelvic sidewall infiltration at the time of recurrence renders pelvic exenteration impossible, making palliative chemotherapy the only possible modality. Lopez–Graniel et al. evaluated the use of neoadjuvant chemotherapy prior to exenteration techniques in this subset of patients aiming to shrink the tumor and therefore allow for subsequent surgery. Nine out of 17 patients (53%) who responded to platinum-based chemotherapy underwent pelvic exenteration, and 4 of them achieved pathologic complete response [17]. An improved median survival of 32 months was observed. In addition, Höckel has performed the laterally extended endopelvic resection (LEER) in 100 patients, among whom 63 were cervical cancer recurrences. LEER is a highly complex surgical procedure with many restrictions regarding its application (good performance status, no comorbidities, curative intent with probability of achieving it, tumor smaller than 5 cm). However, 70% of the patients suffered major surgical-associated morbidities [18].

Isolated Para-aortic Recurrence

Isolated para-aortic recurrence after definitive treatment of cervical cancer ranges from 2% to 12% associated with dismal prognosis [19]. The highest rates of 5-year survival have been reported with the concurrent use of chemoradiation. Chou et al. reported a 5-year OS of 51.2% for women treated with chemoradiotherapy versus 0% for those treated either with chemotherapy or irradiation alone [20]. Furthermore, asymptomatic patients seem to fare much better in cases of isolated para-aortic failures. Irradiation and concurrent cisplatin chemotherapy at a dose of 40 mg/m^2 every week during irradiation had a favorable influence on 5-year OS [19].

Chemotherapy for Recurrent or Metastatic Uterine Cervical Cancer

For the vast majority of patients with recurrent uterine cervical cancer, palliative chemotherapy remains the only treatment modality. The most active agent has historically considered to be cisplatin. However, due to the location of the disease, renal function may already be affected. In addition, the irradiated fields have a lower drug distribution and concentration limiting the effects of cisplatin. Furthermore, myelotoxicity must be taken into consideration due to potential former irradiation therapy.

Combination chemotherapy in the first-line setting has been established as the gold standard for recurrent or metastatic cervical cancer. Many phase II trials conducted have investigated the role of carboplatin, topotecan, paclitaxel, docetaxel, gemcitabine, vinorelbine, irinotecan, ifosfamide, as well as pemetrexed with lower response rates (RR) compared to cisplatin [21–27]. In addition, previously irradiated areas appear to have lower RR.

A randomized phase III trial assigned 294 stage IV patients to receive either cisplatin monotherapy (50 mg/m^2) or the combination of topotecan (0.75 mg/m^2, days 1–3) and cisplatin (50 mg/m^2, day 1) every 3 weeks. Higher RR (27 vs 13%), improved DFI (4.6 vs 2.9 months), and longer median OS (9.4 vs 6.5 months) achieved in the combination arm designated this randomized phase III trial as the first with survival benefit in favor of combination therapy at the expense of more hematological toxicities [28].

The Gynecologic Oncology Group (GOG) 204 trial compared the doublets of cisplatin plus paclitaxel, cisplatin plus vinorelbine, and cisplatin plus gemcitabine with the combination of cisplatin plus topotecan [29]. No significant differences in survival among the different arms were observed with a trend favoring the cisplatin-paclitaxel doublet. This combination also provided better results regarding patients' quality of life. The median OS for the reference cisplatin–paclitaxel regimen was 12.87 months as compared to 9.99 for the cisplatin–vinorelbine combination, 10.28 for the cisplatin–gemcitabine arm, and 10.25 for the combination of cisplatin–topotecan arm. The median DFI was 5.82 months in the cisplatin–paclitaxel arm, 3.98 in the cisplatin–vinorelbine arm, 4.70 in the cisplatin–gemcitabine arm, and 4.57 in the cisplatin–topotecan arm (Table 11.1). Overall, the combinations of cisplatin either with topotecan, gemcitabine, or vinorelbine were not superior in terms of RR, OS, and DFI compared to cisplatin plus paclitaxel. Prognostic factor analysis resulted that age was not a significant factor contrary to performance status which appeared to be the strongest prognostic factor for both DFI and OS.

Pemetrexed, a third-generation antifolate, combined with cisplatin demonstrated activity in the treatment of recurrent uterine cervical cancer [44]. Fifty-four patients with advanced, persistent, or recurrent disease were treated with the combination in a phase II trial [30]. Objective tumor response was assessed including partial and complete responses; 26% of the patients finally received more than nine cycles. One patient achieved complete response and 16 partial responses with a total overall RR of 31%. In previously non-irradiated areas, the RR was 38%. Median DFI and OS were 5.7 and 12.3 months, respectively, with acceptable tolerability (Table 11.1).

Table 11.1 Platinum-based combination regimens for recurrent/advanced cervical cancer

Author/reference	Year of publication	Agent	N	ORR (%)	PFS (months)	OS (months)
Long et al. [28]	2005	Cisplatin	146	13	2.9	6.5
		Cisplatin–topotecan	147	27	4.6	9.4
Monk et al. [29]	2009	Cisplatin–paclitaxel	103	29.1	5.8	12.9
		Cisplatin–vinorelbine	108	25.9	4.0	10.0
		Cisplatin–gemcitabine	112	22.3	4.7	10.3
		Cisplatin–topotecan	111	23.4	4.6	10.3
Miller et al. [30]	2014	Cisplatin–pemetrexed	54	31	5.7	12.3
Bloss et al. [31]	2002	Cisplatin–ifosfamide	303	32	4.6	8.5
		Cisplatin–ifosfamide–bleomycin		31	5.1	8.4
Choi et al. [32]	2006	TIP	53	46.7	8	19
van Luijk et al. [33]	2007	BEMP	161	45	NS	NS
Tewari et al. [34]	2014	Cisplatin–paclitaxel ± bevacizumab	229	38.9	7.6	15.0
		Cisplatin–topotecan ± bevacizumab	223	28.7	5.7	12.5
		Cisplatin–paclitaxel	114	21.4	NS	14.3
		Cisplatin–paclitaxel + bevacizumab	115	43.4	NS	17.6
Moore et al. [35]	2004	Cisplatin	280	19	2.8	8.8
		Cisplatin–paclitaxel		36	4.8	9.7
Kitagawa et al. [36]	2012	Cisplatin–paclitaxel	121	NS	6.9	18.3
		Carboplatin–paclitaxel	123		6.2	17.5
Zanetta et al. [37]	1999	TIP	45	67	NS	6–13
Dimopoulos et al. [38]	2002	TIP	60	46	8.3	18.6
Omura et al. [39]	1997	Cisplatin	454	18	3.2	6.1
		Cisplatin–ifosfamide		31	4.6	7.1
Vermorken et al. [40]	2001	Cisplatin	144	25	4.5	9.3
		BEMP	143	42	5.3	10.1
Mannel et al. [41]	2000	Cisplatin and pentoxifylline	44	9	NS	6
Vermorken et al. [42]	2000	VBMP	50	40	4,4	8,6
Wagenaar et al. [43]	2001	Cisplatin–mitomycin-C	33	42	5	11.2

NS not stated, *OS* overall survival, *ORR* objective response rate, *PFS* progression-free survival, *TIP* paclitaxel, ifosfamide, and cisplatin, *BEMP* bleomycin, vindesine (Eldisine), mitomycin C, and cisplatin, *VBMP* vincristine, bleomycin, mitomycin C, and cisplatin

Three or four drug combinations based on cisplatin salts have not been associated with better clinical responses compared to cisplatin-based doublets or cisplatin monotherapy [31–33] (Table 11.1). Nevertheless, combination therapy with ifosfamide, paclitaxel, and cisplatin (TIP) demonstrated higher pathological optimal RR compared to the cisplatin–ifosfamide doublet in patients with locally advanced squamous cell cervical carcinoma treated in the neoadjuvant setting [45] (Table 11.1). On the other hand, phase II trials have demonstrated RR of 46–67% with the TIP regimen in patients with persistent or recurrent disease which is in accordance with RR achieved with cisplatin-based doublets [31]. Patients with excellent performance status, non-squamous tumors, and recurrent disease only outside previously irradiated fields exhibited the highest RR which were not always statistically significant (Table 11.1).

Beyond First-Line Chemotherapy

After disease progression is documented in patients already treated with platinum-based regimens, inclusion in clinical trials or best supportive care measures are acceptable choices. However treatment in the second-line setting is also an option although the use of single agents has demonstrated rather modest efficacy (Table 11.2). Partial responses with short duration have been documented with a median OS not exceeding 15.5 months. In previously non-irradiated patients, the observed RR was higher. Cisplatin remains an option as active monotherapy with a RR ranging between 13% and 23% [28]. Irinotecan is also a reasonable choice provided that the RR reached 21–24% [48].

Furthermore, several phase II studies have come to equivocal results regarding the use of fluoropyrimidines, in particular capecitabine beyond first line [49, 50]. However, the oral fluoropyrimidine S-1 has been associated with remarkable activity [51]. The reported RR for patients who have been treated with platinums in the first-line setting, including chemoradiotherapy, was 31.8% with a median DFI and OS of 5.2 and 15.4 months, respectively. Currently, a randomized phase III study is evaluating the role of tegafur/gimeracil/oteracil (S-1) in combination with cisplatin versus cisplatin monotherapy in patients with recurrent or metastatic cervical cancer [71]. The use of topotecan as a second-line treatment resulted in RR ranging between 10% and 18% [52, 53], whereas vinorelbine and pemetrexed produced RR ranging between 7–14% [25] and 14–15%, respectively [54]. Minimal activity has also been documented with the use of gemcitabine and docetaxel estimated at 4.5–8% [24] and 9% [23], respectively.

It is of notice that patients who still retain a good performance status with no severe comorbidities could be offered even a third-line therapy. In current literature there are no data for this population. McLachlan et al. have recently reported the results of a series of patients treated with second-line systemic therapy for recurrent or metastatic cervical cancer. Their results provide the information that a large proportion of women will eventually receive more than one line of systemic therapy. The median OS after the induction of second-line treatment was 9.3 months (95% confidence interval [CI], 6.4–12.5); nevertheless, there was a significant difference in OS according to DFI using 6-month and 12-month cutoff values [72].

Table 11.2 Second-line single agents for recurrent/advanced cervical cancer

Author/reference	Year of publication	Agent	N	ORR (%)	PFS (months)	OS (months)
Garcia et al. [23]	2007	Docetaxel	27	8.7	3.8	7.0
Schilder et al. [24, 46]	2005	Gemcitabine	22	4.5	2.1	6.5
	2000	Gemcitabine	24	8	1.9	4.9
Muggia et al. [25]	2005	Vinorelbine	28	7.1	NS	NS
Sutton et al. [27, 47]	1993	Ifosfamide	39	15	NS	4.2
	1989	Ifosfamide	27	11	NS	NS
Verschraegen et al. [48]	1997	Irinotecan	42	21	NS	NS
Look et al. [49]	2008	Capecitabine	21	0	NS	NS
Lorvidhaya et al. [50]	2010	Capecitabine	45	2	4.1	9.3
Katsumata et al. [51]	2011	S-1	36	31.8	5.2	15.4
Coronel et al. [52]	2009	Topotecan	18	NS	3.5	7.0
Fiorica et al. [53]	2009	Topotecan	25	NS	2.4	6.2
Lorusso et al. [54]	2010	Pemetrexed	43	13.9	2.5	8.8
Thigpen [55]	2003	Cisplatin	190	23	NS	NS
		Ifosfamide	35	14–40	NS	NS
Takeuchi et al. [56]	1991	Irinotecan	55	24	NS	NS
Garcia et al. [57]	2007	Capecitabine	26	15.4	2.9	5.9
Bookman et al. [58]	2000	Topotecan	45	12.5	2.1	6.6
Noda et al. [59]	1996	Topotecan	22	18	NS	NS
Abu-Rustum et al. [60]	2000	Topotecan	12	17	NS	NS
Nascimento de Oliveira et al. [61]	2013	Topotecan	21	10	2.93	4.66
Muggia et al. [62]	2004	Vinorelbine	44	13.7	NS	NS
Miller et al. [63]	2008	Pemetrexed	29	15	3.1	7.4
Thigpen et al. [64]	1981	Cisplatin	12	17	NS	NS
Rose et al. [65–67]	2006	Pegylated liposomal doxorubicin	26	11	NS	NS
	1996	Altretamine	29	0	NS	4.6
	1998	Etoposide	24	8	NS	3.7
Curtin et al. [68]	2001	Paclitaxel	41	32	NS	7.3
Vermorken et al. [69]	1991	DAC	15	0	NS	NS
van der Burg et al. [70]	1992	4'-epidoxorubicin	24	4.2	3.2	NS

NS not stated, *OS* overall survival, *ORR* objective response rate, *PFS* progression-free survival, *DAC* 5-aza-2'-deoxycytidine

Recently Manders et al. reported the results of a single-institution review regarding third-line therapy for patients with recurrent or metastatic cervical cancer [73]. The reported RR was 10% with 27% of patients achieving stable disease. The estimated DFI was 3.8 months and the OS 7.4 months at the expense of toxicities. Grade 3 or 4 adverse effects and toxicities were documented in 57% of the patients. Thus, when it comes down to treat beyond second line, quality of life and patient's costs must be taken into consideration. Finally, since there is no established current choice for treatment beyond first line, there is an imperative need for research in this field in order to enhance the armory against cervical cancer in this setting.

The Role of Platinums Beyond First Line

When treating a patient with recurrent or metastatic cervical cancer, prior exposure to platinum salts may affect the results of next-line therapy. Although there are reports of RR between 31% and 36% with the use of platinums, the median OS of patients does not exceed 19 months [28, 29, 31] (Table 11.1). The impact of prior platinum exposure has been investigated. Tewari and Monk reported that the RR in platinum-naïve patients was 20% with cisplatin monotherapy, 39% with the combination of cisplatin plus topotecan, and 37% when cisplatin is combined with paclitaxel. However, after prior platinum therapy, the RR was estimated to be between 5% and 8% for cisplatin monotherapy and 15% and 32% with the combinations of cisplatin–topotecan and cisplatin–paclitaxel, respectively [74]. Thus, a history of prior platinum exposure may be indicative of poorer results beyond first-line treatment and that platinum combination may be preferred options in this population.

Antiangiogenesis

Neovascularization of the tumor is tightly correlated to the extent of disease and patient's survival. Strong expression of the endothelial cell marker CD31 along with high microvessel density is indicative of poorer survival. Both invasive cervical carcinomas and high-grade dysplasia are associated with increased expression of VEGF and hypoxia-inducible factor (HIF-1a). When hypoxic conditions prevail, HPV oncoproteins form a complex with HIF-1a and promote VEGF expression as well as hypoxia-induced HIF-1a [75].

Bevacizumab, a recombinant humanized monoclonal antibody that binds to the vascular endothelial growth factor A (VEGF-A), has antiangiogenic action. To date bevacizumab is approved for the treatments of a wide range of neoplasms such as lung, breast, ovarian, colorectal, and renal cell cancers. GOG 240, a randomized phase III four-arm clinical trial, investigated the role of bevacizumab in combination with either platinum or non-platinum-based regimens (Table 11.3) [34]. Patients were randomly assigned to one of the four arms. Control arm consisted

Table 11.3 Non-platinum-based combination regimens for recurrent/advanced cervical cancer

Author/ reference	Year of publication	Agent	N	ORR (%)	PFS (months)	OS (months)
Tewari et al. [34]	2014	Topotecan–paclitaxel	111	24.3	NS	12.7
		Topotecan–paclitaxel + bevacizumab	112	42	NS	16.2
Look et al. [76, 77]	1996	5-FU and leucovorin	45	9	NS	NS
	1998	Isotretinoin and interferon alfa	34	3	NS	3.9

NS not stated, *OS* overall survival, *ORR* objective response rate, *PFS* progression-free survival

of cisplatin–paclitaxel and the non-platinum combination of topotecan plus paclitaxel. Each of these arms was evaluated with or without concurrent administration of bevacizumab. The non-platinum doublet was associated with a higher risk of progression, while no adverse impact on OS was observed. Treatment with cisplatin–paclitaxel and bevacizumab, compared to cisplatin–paclitaxel alone, provided an estimated hazard ratio (HR) for death 0.68, while RR were 50% and 45%, respectively. The combination of topotecan–paclitaxel plus bevacizumab had calculated HR for death 0.74. The estimated RR were 47% and 27% for the bevacizumab-containing regimen and the chemotherapy-alone arm, respectively.

In addition, lesions located in previously irradiated pelvic areas also responded to the use of bevacizumab. Main adverse effects were hypertension, the formation of genitourinary or gastrointestinal fistulas, and thromboembolic events. In this trial, more than 70% of the patients had already been exposed to prior platinum therapy making it difficult to evaluate the role of bevacizumab in the neoadjuvant setting prior to surgery. The role of bevacizumab has also been investigated in combination with standard chemoradiotherapy in bulky IB–IIIB disease, reporting a DFI of 68.7%; nevertheless, Zingelboim et al. have reported that besides the remarkable activity of bevacizumab when added to cisplatin–topotecan combination, merely 80% of the patients suffered adverse events [78, 79].

Apart from bevacizumab, cediranib has also achieved efficient results when used for advanced cervical cancer. Cediranib is a tyrosine kinase inhibitor (TKI) targeting VEGF 1, 2, and 3 and c-kit. A randomized placebo-controlled phase II trial, CIRCCa, investigated the role of cediranib in patients with metastatic or recurrent cervical cancer in the first-line setting when added to carboplatin and paclitaxel combination. There was a significant improvement in DFI with the triplet combination (8.1 versus 6.7 months; HR = 0.58; P = 0.032) without any impact on OS even though a number of long-term survivors were identified [72].

Bevacizumab-containing regimens for use in metastatic or recurrent cervical cancer were associated with a reduced hazard of disease progression and prolonged OS even in previously irradiated pelvis. On August 14, 2014, the Food and Drug Administration (FDA) approved bevacizumab in combination with paclitaxel and either cisplatin or topotecan for the treatment of persistent, recurrent, or metastatic uterine cervical cancer based on the results of the GOG 240 trial. Thus, bevacizumab is the only agent approved in the last years for the treatment of cervical

cancer. Due to its effectiveness, prolongation of survival is achieved for patients with metastatic or recurrent cervical cancer. In this way, a larger amount of patients will be candidates for further line therapies, where more research is needed.

Epidermal Growth Factor Receptor (EGFR) Inhibition

The EGFR is a tyrosine kinase growth factor receptor belonging to the ErbB family. Upon ligand binding, subsequent homo- and heterodimer formation leads to the activation of several pathways which contribute to cell growth and proliferation. Overall the expression of EGFR in cervical cancer varies from 6% to 90% and together with the overexpression of human epidermal growth factor receptor 2 (HER2) is related with dismal prognosis [80, 81]. Belone et al. documented that primary cervical cancer lines from tumor biopsies and sites of disease recurrence express EGFR [80]. In addition, high-grade intraepithelial neoplasia cells also express EGFR. Consequently, there seems to be an association between HPV infection and EGFR expression, but correlation with specific subtypes of HPV virus has not been found.

Cetuximab, a chimeric human-murine monoclonal antibody targeting EGFR, administered with cisplatin in persistent or recurrent cervical cancer was associated with increased toxicity and an estimated RR of 9% [82] (Table 11.4). It has also been investigated with the combination of cisplatin plus topotecan with an estimated OS of 6.8 months and stable disease in 32% of the patients; yet, major grade 3 or 4 toxicities were detected [83] (Table 11.4). Previous treatments and poor performance status may contribute to the incidence of adverse effects. At the 2015 American Society of Clinical Oncology (ASCO) annual meeting, the results of a phase II trial were reported comparing carboplatin plus paclitaxel with or without cetuximab. No gain in DFI or OS was observed with the triplet combination [99]. Finally, nimotuzumab has been reported to have modest activity in combination with gemcitabine in 17 patients beyond first line with refractory or progressive cervical cancer [84] (Table 11.4).

Erlotinib, an EGFR TKI, has also been evaluated by the GOG in patients with squamous disease relapse; no objective responses were observed, and only one patient (4%) achieved a PFI longer than 6 months [85]. Gefitinib has also demonstrated minimal activity as well as imatinib [86, 87] (Table 11.4). In the studies investigating these TKIs, no objective responses were documented. Gefitinib achieved stabilization in 20% of the 30 evaluated patients with disease resistant to standard treatment. The rationale for imatinib was based on the expression of platelet-derived growth factor receptors (PDGFR). Overall, poor results of EGFR TKI activity are expected due to the lack of mutations in exons 18–21 and the lack of c-kit in cervical cell lines [100, 101].

Pazopanib, a multi-TKI inhibitor which targets VEGF 1, 2, and 3, PDGFR-a and PDGFR-b, and c-kit, has been evaluated either as monotherapy or in combination with lapatinib, an anti-EGFR, and HER2 TKI. In this study the activity of pazopanib was illustrated based on the prolonged DFI (HR = 0.66; P = 0.013),

Table 11.4 Second-line novel agents for recurrent/advanced cervical cancer

Author/ reference	Year of publication	Agent	N	ORR (%)	PFS (months)	OS (months)
Farley et al. [82]	2011	Cisplatin–cetuximab	44	9	NS	NS
Kurtz et al. [83]	2009	Cisplatin–topotecan–cetuximab	19	32	5.7	7.3
Cetina et al. [84]	2015	Nimotuzumab + gemcitabine or cisplatin	17	0	5.4	9.8
Schilder et al. [85]	2009	Erlotinib	28	0	1.87	4.96
Goncalves et al. [86]	2008	Gefitinib	28	0	1.23	3.56
Candelaria et al. [87]	2009	Imatinib	12	0	NS	NS
Monk et al. [88]	2010	Lapatinib	78	5	4.3	9.8
		Pazopanib	74	9	4.5	12.6
Tinker et al. [89]	2013	Temsirolimus	38	3	3.52	NS
Stevanović et al. [90]	2015	HPV-TILs	9	33.3	NS	NS
Petit et al. [91]	2014	Cisplatin–ADXs11–01	110	11	NS	NS
Frenel et al. [92]	2016	Pembrolizumab	23	17	2	9
Monk et al. [93]	2009	Bevacizumab	46	10.9	3.4	7.29
Mackay et al. [94]	2010	Sunitinib	19	0	3.5	NS
Santin et al. [95]	2011	Cetuximab	38	0	1.97	6.7
Hertlein et al. [96]	2011	Cetuximab	5	0	NS	8.6
Lheureux et al. [97]	2015	Ipilimumab	42	8.8	2.5	NS
Burotto et al. [98]	2015	Ixabepilone	41	9.7	2.3	5.8

NS not stated, *OS* overall survival, *ORR* objective response rate, *PFS* progression-free survival, *TILs* tumor-infiltrating T-cells

OS (HR = 0.67; P = 0.045), and the acceptable toxicity. DFI was shorter in the cohort of patients treated with lapatinib compared to those who received pazopanib (17.1 and 18.1 weeks, respectively) (Table 11.4). Median OS was improved by 11.6 weeks (50.7 versus 39.1), while RR were 9% and 5%, respectively. The combination arm demonstrated unacceptable toxicities leading to discontinuation of treatment, while poor results of the combination may be due to the antagonistic interaction between the two agents [88].

PI3K/AKT/mTOR Inhibitors

The activation PI3K/AKT/mTOR pathway is a common feature in nearly all HPV-associated squamous cell carcinomas of the cervix making it a potential target for new therapeutic strategies. Interestingly enough, inhibition of mechanistic target of rapamycin (mTOR) actively blocks cervical cancer growth [102, 103]. When phosphorylated by mTOR, the overexpressed downstream regulators P70S6K and 4EBPI induce translation of mRNA-encoding proteins involved in cell cycle proliferation [104]. Natural inhibition of mTOR is caused by rapamycin, so rapalogs, the class of agents which includes temsirolimus and everolimus, can target the mTOR pathway. They inhibit MTORC1, part of the mTOR complex, and consequently block AKT. Temsirolimus can be used as a single agent in a neoadjuvant setting and may reduce the dose of chemotherapy or radiotherapy required for local disease. It can also delay tumor recurrence after prior surgery with or without additional chemoradiotherapy.

In a two-stage phase II study, the activity of temsirolimus was evaluated in patients with metastatic or recurrent cervical carcinoma (Table 11.4). One out of the 38 participants achieved partial response, and 19 experienced stable disease with a median duration of 6.5 months. The estimated 6-month DFI rate and median DFI were 28% [95% CI, 14–34%] and 3.52 months [95% CI, 1.81–4.70], respectively, demonstrating no activity in patients with recurrent or metastatic cervical cancer [89]. Currently, ongoing trials are evaluating the role of mTOR inhibition in patients with locally advanced cervical cancer in combination with the gold standard treatments.

Poly[ADP-Ribose] Polymerase (PARP) Inhibition

PARP enzymes interfere with DNA base repairment, playing a crucial role in genetic stability. Inhibition of PARP enzymes leads to cell inability to repair DNA damage sites. To date two PARP inhibitors, olaparib and veliparib, have shown promising results in BRCA1- or BRCA2-deficient cells leading to FDA approval of olaparib for the treatment of recurrent ovarian cancer in BRCA-mutated patients [105]. The role of PARP inhibition in cervical cancer has also been examined. Among 27 patients with recurrent or progressive cervical cancer who were treated with the combination of topotecan plus veliparib, two partial responses were reported, and four patients had disease progression more than 6 months after therapy induction [106]. Currently a single-arm phase I–II trial is ongoing investigating the potentials of cisplatin plus paclitaxel in combination with veliparib in patients with metastatic or recurrent cervical cancer; phase I part results showed an overall RR of 60% [107].

Therapeutic Vaccines

Immunotherapy is an emerging modality in oncology. As aforementioned, cervical cancer holds a strong association with HPV infection. Two HPV16 proteins, E6 and E7, are consistently expressed in tumor cells promoting replication and

immortalization. In the era of immunotherapy, the role of therapeutic vaccination is gaining interest. Most of the therapeutic vaccines tested in cervical cancer target one or both E6 and E7 viral oncoproteins. Protein vaccination is considered the most popular type of HPV vaccine due to its safety and absence of human leukocyte antigen restriction [108].

A recent study assessed the HPV-targeted tumor-infiltrating lymphocytes therapy in patients with metastatic cervical cancer beyond first line [90] (Table 11.4). T-cells were collected from the tumor and cultured. The most reactive cell cultures for E6 and E7 were evaluated, and expanded T-cells were infused into the patient. The HPV reactivity of T-cells in the infusion product correlated with clinical response. In addition, the frequency of HPV-reactive T-cells in peripheral blood 1 month after the therapy was also associated with clinical response. Three out of nine patients who received tumor-infiltrating lymphocytes had evidence of complete or partial response.

Furthermore, live-attenuated *Listeria monocytogenes*-based vaccine (ADXS11-01) has been tested in a phase II study [91]. Patients, who had already undergone chemotherapy, radiotherapy, or both, were randomized to either 3 or 4 doses of the vaccine in combination with cisplatin. The achieved 18- and 12-month survival was 28% and 36%, respectively, while the overall RR was 11% with an average duration of 10.5 months after cycle 1. Prior therapy, performance status, and the combination with cisplatin had no impact on survival or response. However, further studies are needed in order to determine the optimal dosage and number of cycles of ADXS11-01 administration.

Another investigated vaccine, bryostatin-1, which consists of a lactone binding to Toll-like receptor 4, upon administration promotes dendritic cell and lymphocyte activation in vitro. However, in a GOG phase II study, the combination of bryostatin-1 with cisplatin in patients with metastatic or recurrent cervical cancer showed poor activity [109].

Immune Checkpoint Inhibition

Tumor cells have the ability to evade the immune system maintaining proliferation and growth advantage. Immunotherapy that inhibits critical checkpoints of the immune system offers the ability to engage immunoreactivity against cancer cells. Ipilimumab, nivolumab, and pembrolizumab are currently under investigation in clinical trials. Both anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) ipilimumab and anti-programmed cell death 1 (PD-1) nivolumab are monoclonal antibodies that target and block CTLA-4 and PD-1 receptors, respectively, which are negative regulatory molecules of T-cell activation [110]. An open-label, single-arm phase I trial is ongoing evaluating the impact of ipilimumab administration in women with cervical cancer who have already undergone chemoradiotherapy for locally advanced disease [111]. In addition, nivolumab is also under investigation in a phase II trial in patients with recurrent and metastatic cervical cancer [112].

The KEYNOTE-028 multicohort study was a phase IB study evaluating the safety and efficacy of pembrolizumab in patients with programmed death-ligand 1 (PD-L1)-positive tumors after failure of prior systemic therapy. The overall RR in the 23 evaluable patients with unresectable or metastatic cervical cancer was 17% with a median duration of response of 26 weeks [92] (Table 11.4). Durvalumab is under investigation in patients with advanced solid tumors including a cohort of patients with HPV-positive cancer. At ASCO 2016, two case reports were presented regarding patients with metastatic cervical cancer. They received REGN2810, a fully human anti-PD-1 monoclonal antibody in combination with radiotherapy. The patients achieved complete and partial response, documented 16 and 12 months after the therapy, respectively [113].

Other Investigational Agents

In the field of cervical cancer therapeutics, research is ongoing in order to elucidate the role of new emerging therapies. The use of adenoviruses as live vectors which encode wild-type p53 has given promising results in vitro [114]. Another potential agent, geldanamycin, a heat shock protein inhibitor (HSP90 inhibitor), has also shown efficacy in cervical cancer cell lines in combination with radiotherapy [115]. At the moment a randomized double-blind phase II trial is ongoing investigating the administration of cisplatin plus topotecan and MK1775 in patients with metastatic or recurrent cervical cancer [116]. MK1775 is a WEE1 inhibitor which promotes premature cell mitosis and subsequent cell death [117]. Furthermore, DNA methyltransferase inhibitors, such as azacitidine and decitabine, are being evaluated in cervical cancer therapeutics. In particular, the combination of decitabine plus cisplatin has been evaluated in a phase II study in patients with metastatic cervical cancer with 38.1% of them achieving partial response and 23.8% stable disease [118]. Finally, the results from a recent study involving next-generation sequencing, in situ hybridization, and immunohistochemistry of 592 cervical cancer specimens may bring to spotlight novel agents for patients who have progressed after first-line therapy through clinical trials [119].

Conclusions

Patients with recurrent or metastatic uterine cervical cancer consist a high-risk population due to the fact that no specific therapy has been established for second-line treatment. After exposure to platinum-based regimens, a significant proportion of this population will still retain good performance status and thus be candidates for further treatment interventions. In fact, due to the incorporation of antiangiogenesis in first-line setting, even more patients will eventually survive to the point where the clinician must proceed with the decision of second- and even third-line treatment. Research and participation in clinical trials are the only ways to expand the current armory against recurrent or metastatic cervical cancer.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86.
2. Pimple S, Mishra G, Shastri S. Global strategies for cervical cancer prevention. Curr Opin Obstet Gynecol. 2016;28:4–10.
3. Bryan JT, Buckland B, Hammond J, Jansen KU. Prevention of cervical cancer: journey to develop the first human papillomavirus virus-like particle vaccine and the next generation vaccine. Curr Opin Chem Biol. 2016;32:34–47.
4. Pfaendler KS, Tewari KS. Changing paradigms in the systemic treatment of advanced cervical cancer. Am J Obstet Gynecol. 2016;214(1):22–30.
5. Li H, Wu X, Cheng X. Advances in diagnosis and treatment of metastatic cervical cancer. J Gynecol Oncol. 2016;27(4):e43.
6. Quinn MA, Benedet JL, Odicino F, Maisonneuve P, Beller U, Creasman WT, Heintz AP, Ngan HY, Pecorelli S. Carcinoma of the cervix uteri. FIGO 26th annual report on the results of treatment in gynecological cancer. Int J Gynaecol Obstet. 2006;95(Suppl 1):S43–103.
7. Dornhöfer N, Höckel M. New developments in the surgical therapy of cervical carcinoma. Ann N Y Acad Sci. 2008;1138:233–52.
8. Zola P, Fuso L, Mazzola S, Piovano E, Perotto S, Gadducci A, Galletto L, Landoni F, Maggino T, Raspagliesi F, Sartori E, Scambia G. Could follow-up different modalities play a role in asymptomatic cervical cancer relapses diagnosis? An Italian multicenter retrospective analysis. Gynecol Oncol. 2007;107(1 Suppl 1):S150–4.
9. Perez CA, Grigsby PW, Camel HM, Galakatos AE, Mutch D, Lockett MA. Irradiation alone or combined with surgery in stage IB, IIA, and IIB carcinoma of uterine cervix: update of a nonrandomized comparison. Int J Radiat Oncol Biol Phys. 1995;31(4):703–16.
10. Piura B, Rabinovich A, Friger M. Recurrent cervical carcinoma after radical hysterectomy and pelvic lymph node dissection: a study of 32 cases. Eur J Gynaecol Oncol. 2008;29(1):31–6.
11. Friedlander M, Grogan M, U.S. Preventative Services Task Force. Guidelines for the treatment of recurrent and metastatic cervical cancer. Oncologist. 2002;7(4):342–7.
12. Goldberg GL, Sukumvanich P, Einstein MH, Smith HO, Anderson PS, Fields AL. Total pelvic exenteration: the Albert Einstein College of Medicine/Montefiore Medical Center Experience (1987 to 2003). Gynecol Oncol. 2006;101(2):261–8.
13. Fleisch MC, Pantke P, Beckmann MW, Schnuerch HG, Ackermann R, Grimm MO, Bender HG, Dall P. Predictors for long-term survival after interdisciplinary salvage surgery for advanced or recurrent gynecologic cancers. J Surg Oncol. 2007;95(6):476–84.
14. Panici PB, Angioli R, Plotti F, Muzii L, Zullo MA, Manci N, Palaia I, Galluci M. Continent ileocolonic urinary diversion (Rome pouch) for gynecologic malignancies: technique and feasibility. Gynecol Oncol. 2007;107(2):194–9.
15. Jover R, Lourido D, Gonzalez C, Rojo A, Gorospe L, Alfonso JM. Role of PET/CTin the evaluation of cervical cancer. Gynecol Oncol. 2008;110(3 Suppl 2):S55–9.
16. Green AE, Escobar PF, Neubaurer N, Michener CM, Vongruenigen VE. The Martius flap neovagina revisited. Int J Gynecol Cancer. 2005;15(5):964–6.
17. Lopez-Graniel C, Dolores R, Cetina L, Gonzalez A, Cantu D, Chanona J, Uribe J, Candelaria M, Brom R, de la Garza J, Duenas-Gonzalez A. Pre-exenterative chemotherapy, a novel therapeutic approach for patients with persistent or recurrent cervical cancer. BMC Cancer. 2005;5:118.
18. Höckel M. Laterally extended endopelvic resection (LEER) – principles and practice. Gynecol Oncol. 2008;111(2 Suppl):S13–7.
19. Singh AK, Grigsby PW, Rader JS, Mutch DG, Powell MA. Cervix carcinoma, concurrent chemoradiotherapy, and salvage of isolated paraaortic lymph node recurrence. Int J Radiat Oncol Biol Phys. 2005;61(2):450–5.

20. Chou HH, Wang CC, Lai CH, Hong JH, Ng KK, Chang TC, Tseng CJ, Tsai CS, Chang JT. Isolated paraaortic lymph node recurrence after definitive irradiation for cervical carcinoma. Int J Radiat Oncol Biol Phys. 2001;51(2):442–8.
21. Muderspach LI, Blessing JA, Levenback C, Moore JL Jr. A phase II study of topotecan in patients with squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. Gynecol Oncol. 2001;81(2):213–5.
22. Kudelka AP, Winn R, Edwards CL, Downey G, Greenberg H, Dakhil SR, Freedman RS, LoCoco S, Umbreit J, Delmore JE, Arbuck S, Loyer E, Gacrama P, Fueger R, Kavanagh JJ. An update of a phase II study of paclitaxel in advanced or recurrent squamous cell cancer of the cervix. Anti-Cancer Drugs. 1997;8(7):657–61.
23. Garcia AA, Blessing JA, Vaccarello L, Roman LD, Gynecologic Oncology Group Study. Gynecologic Oncology Group study: phase II clinical trial of docetaxel in refractory squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. Am J Clin Oncol. 2007;30(4):428–31.
24. Schilder RJ, Blessing J, Cohn DE. Evaluation of gemcitabine in previously treated patients with non-squamous cell carcinoma of the cervix: a phase II study of the Gynecologic Oncology Group. Gynecol Oncol. 2005;96(1):103–7.
25. Muggia FM, Blessing JA, Waggoner S, Berek JS, Monk BJ, Sorosky J, Pearl ML. Evaluation of vinorelbine in persistent or recurrent nonsquamous carcinoma of the cervix: a Gynecologic Oncology Group study. Gynecol Oncol. 2005;96(1):108–11.
26. Lhommé C, Fumoleau P, Fargeot P, Krakowski Y, Dieras V, Chauvergne J, Vennin P, Rebattu P, Roche H, Misset JL, Lentz MA, Van Glabbeke M, Matthieu-Boué A, Mignard D, Chevallier B. Results of a European Organization for Research and Treatment of Cancer/Early Clinical Studies Group phase II trial of first-line irinotecan in patients with advanced or recurrent squamous cell carcinoma of the cervix. J Clin Oncol. 1999;17(10):3136–42.
27. Sutton GP, Blessing JA, DiSaia PJ, McGuire WP. Phase II study of ifosfamide and mesna in nonsquamous carcinoma of the cervix: a Gynecologic Oncology Group study. Gynecol Oncol. 1993;49(1):48–50.
28. Long HJ 3rd, Bundy BN, Grendys EC Jr, Benda JA, McMeekin DS, Sorosky J, Miller DS, Eaton LA, Fiorica JV, Gynecologic Oncology Group Study. Randomized phase III trial of cisplatin with or without topotecan in carcinoma of the uterine cervix: a Gynecologic Oncology Group study. J Clin Oncol. 2005;23(21):4626–33.
29. Monk BJ, Sill MW, McMeekin DS, Cohn DE, Ramondetta LM, Boardman CH, Benda J, Cella D. Phase III trial of four cisplatin-containing doublet combinations in stage IVB, recurrent, or persistent cervical carcinoma: a Gynecologic Oncology Group study. J Clin Oncol. 2009;27(28):4649–55.
30. Miller DS, Blessing JA, Ramondetta LM, Pham HQ, Tewari KS, Landrum LM, Brown J, Mannel RS. Pemetrexed and cisplatin for the treatment of advanced, persistent, or recurrent carcinoma of the cervix: a limited access phase II trial of the Gynecologic Oncology Group. J Clin Oncol. 2014;32(25):2744–9.
31. Bloss JD, Blessing JA, Behrens BC, Mannel RS, Rader JS, Sood AK, Markman M, Benda J. Randomized trial of cisplatin and ifosfamide with or without bleomycin in squamous carcinoma of the cervix: a Gynecologic Oncology Group study. J Clin Oncol. 2002;20(7):1832–7.
32. Choi CH, Kim TJ, Lee SJ, Lee JW, Kim BG, Lee JH, Bae DS. Salvage chemotherapy with a combination of paclitaxel, ifosfamide, and cisplatin for the patients with recurrent carcinoma of the uterine cervix. Int J Gynecol Cancer. 2006;16(3):1157–64.
33. van Luijk IF, Coens C, van der Burg ME, Kobierska A, Namer M, Lhomme C, Zola P, Zanetta G, Vermorken JB, Gynecological Cancer Group of the European Organization for Research and Treatment of Cancer. Phase II study of bleomycin, vindesine, mitomycin C and cisplatin (BEMP) in recurrent or disseminated squamous cell carcinoma of the uterine cervix. Ann Oncol. 2007;18(2):275–81.
34. Tewari KS, Sill MW, Long HJ 3rd, Penson RT, Huang H, Ramondetta LM, Landrum LM, Oaknin A, Reid TJ, Leitao MM, Michael HE, Monk BJ. Improved survival with bevacizumab in advanced cervical cancer. N Engl J Med. 2014;370(8):734–43.

35. Moore DH, Blessing JA, McQuellon RP, Thaler HT, Cella D, Benda J, Miller DS, Olt G, King S, Boggess JF, Rocereto TF. Phase III study of cisplatin with or without paclitaxel in stage IVB, recurrent, or persistent squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. J Clin Oncol. 2004;22(15):3113–9.
36. Kitagawa R, Katsumata N, Shibata T, Kamura T, Kasamatsu T, Nakanishi T, Nishimura S, Ushijima K, Takano M, Satoh T, Yoshikawa H. Paclitaxel plus carboplatin versus paclitaxel plus cisplatin in metastatic or recurrent cervical cancer: the open-label randomized phase III trial JCOG0505. J Clin Oncol. 2015;33(19):2129–35.
37. Zanetta G, Fei F, Parma G, Balestrino M, Lissoni A, Gabriele A, Mangioni C. Paclitaxel, ifosfamide and cisplatin (TIP) chemotherapy for recurrent or persistent squamous-cell cervical cancer. Ann Oncol. 1999;10(10):1171–4.
38. Dimopoulos MA, Papadimitriou CA, Sarris K, Aravantinos G, Kalofonos C, Gika D, Gourgoulis GM, Efstathiou E, Skarlos D, Bafaloukos D. Combination of ifosfamide, paclitaxel, and cisplatin for the treatment of metastatic and recurrent carcinoma of the uterine cervix: a phase II study of the Hellenic Cooperative Oncology Group. Gynecol Oncol. 2002;85(3):476–82.
39. Omura GA, Blessing JA, Vaccarello L, Berman ML, Clarke-Pearson DL, Mutch DG, Anderson B. Randomized trial of cisplatin versus cisplatin plus mitolactol versus cisplatin plus ifosfamide in advanced squamous carcinoma of the cervix: a Gynecologic Oncology Group study. J Clin Oncol. 1997;15(1):165–71.
40. Vermorken JB, Zanetta G, De Oliveira CF, van der Burg ME, Lacave AJ, Teodorovic I, Boes GH, Colombo N. Randomized phase III trial of bleomycin, vindesine, mitomycin-C, and cisplatin (BEMP) versus cisplatin (P) in disseminated squamous-cell carcinoma of the uterine cervix: an EORTC Gynecological Cancer Cooperative Group study. Ann Oncol. 2001;12(7):967–74.
41. Mannel RS, Blessing JA, Boike G. Cisplatin and pentoxifylline in advanced or recurrent squamous cell carcinoma of the cervix: a phase II trial of the Gynecologic Oncology Group. Gynecol Oncol. 2000;79(1):64–6.
42. Vermorken JB, Mangioni C, Pecorelli S, Van Der Burg ME, Van Oosterom AT, Ten Bokkel Huinink WW, Rotmensz N, Dalesio O. Phase II study of vincristine, bleomycin, mitomycin C and cisplatin (VBMP) in disseminated squamous cell carcinoma of the uterine cervix. Int J Gynecol Cancer. 2000;10(5):358–65.
43. Wagenaar HC, Pecorelli S, Mangioni C, van der Burg ME, Rotmensz N, Anastasopoulou A, Zola P, Veenhof CH, Lacave AJ, Neijt JP, van Oosterom AT, Einhorn N, Vermorken JB. Phase II study of mitomycin-C and cisplatin in disseminated, squamous cell carcinoma of the uterine cervix. A European Organization for Research and Treatment of Cancer (EORTC) Gynecological Cancer Group study. Eur J Cancer. 2001;37(13):1624–8.
44. Miller DS, Tai DF, Obasaju C, Vergote I. Safety and efficacy of pemetrexed in gynecologic cancers: a systematic literature review. Mod Chemother. 2013;2:19–32.
45. Buda A, Fossati R, Colombo N, Fei F, Floriani I, Gueli Alletti D, Katsaros D, Landoni F, Lissoni A, Malzoni C, Sartori E, Scollo P, Torri V, Zola P, Mangioni C. Randomized trial of neoadjuvant chemotherapy comparing paclitaxel, ifosfamide, and cisplatin with ifosfamide and cisplatin followed by radical surgery in patients with locally advanced squamous cell cervical carcinoma: the SNAP01 (Studio Neo-Adjuvante Portio) Italian Collaborative Study. J Clin Oncol. 2005;23(18):4137–45.
46. Schilder RJ, Blessing JA, Morgan M, Mangan CE, Rader JS. Evaluation of gemcitabine in patients with squamous cell carcinoma of the cervix: a phase II study of the Gynecologic Oncology Group. Gynecol Oncol. 2000;76(2):204–7.
47. Sutton GP, Blessing JA, Adcock L, Webster KD, DeEulis T. Phase II study of ifosfamide and mesna in patients with previously-treated carcinoma of the cervix. A Gynecologic Oncology Group study. Investig New Drugs. 1989;7(4):341–3.
48. Verschraegen CF, Levy T, Kudelka AP, Llerena E, Ende K, Freedman RS, Edwards CL, Hord M, Steger M, Kaplan AL, Kieback D, Fishman A, Kavanagh JJ. Phase II study of irinotecan in prior chemotherapy-treated squamous cell carcinoma of the cervix. J Clin Oncol. 1997;15(2):625–31.

49. Look KY, Blessing JA, Michener CM, Rubin S, Ramirez PT, Gynecologic Oncology Group Study. Phase II evaluation of capecitabine in refractory nonsquamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. Int J Gynecol Cancer. 2008;18(4):773–8.
50. Lorvidhaya V, Chitapanarux I, Phromratanapongse P, Kamnerdsupaphon P, Tharavichitkul E, Lertsanguansinchai P, Hsieh CY, Sukthomya V. Phase II study of capecitabine (Ro 09-1978) in patients who have failed first line treatment for locally advanced and/or metastatic cervical cancer. Gan To Kagaku Ryoho. 2010;37(7):1271–5.
51. Katsumata N, Hirai Y, Kamiura S, Sugiyama T, Kokawa K, Hatae M, Nishimura R, Ochiai K. Phase II study of S-1, an oral fluoropyrimidine, in patients with advanced or recurrent cervical cancer. Ann Oncol. 2011;22(6):1353–7.
52. Coronel J, Cetina L, Candelaria M, González-Fierro A, Arias D, Cantu D, Dueñas-González A. Weekly topotecan as second- or third-line treatment in patients with recurrent or metastatic cervical cancer. Med Oncol. 2009;26(2):210–4.
53. Fiorica JV, Blessing JA, Puneky LV, Secord AA, Hoffman JS, Yamada SD, Buekers TE, Bell J, Schilder JM, Gynecologic Oncology Group. A phase II evaluation of weekly topotecan as a single agent second line therapy in persistent or recurrent carcinoma of the cervix: a Gynecologic Oncology Group study. Gynecol Oncol. 2009;115(2):285–9.
54. Lorusso D, Ferrandina G, Pignata S, Ludovisi M, Viganò R, Scalone S, Scollo P, Breda E, Pietragalla A, Scambia G. Evaluation of pemetrexed (Alimta, LY231514) as second-line chemotherapy in persistent or recurrent carcinoma of the cervix: the CERVIX 1 study of the MITO (Multicentre Italian Trials in Ovarian Cancer and Gynecologic Malignancies) Group. Ann Oncol. 2010;21(1):61–6.
55. Thigpen T. The role of chemotherapy in the management of carcinoma of the cervix. Cancer J. 2003;9(5):425–32.
56. Takeuchi S, Dobashi K, Fujimoto S, Tanaka K, Suzuki M, Terashima Y, Hasumi K, Akiya K, Negishi Y, Tamaya T, et al. A late phase II study of CPT-11 on uterine cervical cancer and ovarian cancer. Research Groups of CPT-11 in Gynecologic Cancers. Gan To Kagaku Ryoho. 1991;18(10):1681–9.
57. Garcia AA, Blessing JA, Darcy KM, Lenz HJ, Zhang W, Hannigan E, Moore DH. Phase II clinical trial of capecitabine in the treatment of advanced, persistent or recurrent squamous cell carcinoma of the cervix with translational research: a Gynecologic Oncology Group study. Gynecol Oncol. 2007;104(3):572–9.
58. Bookman MA, Blessing JA, Hanjani P, Herzog TJ, Andersen WA. Topotecan in squamous cell carcinoma of the cervix: a phase II study of the Gynecologic Oncology Group. Gynecol Oncol. 2000;77(3):446–9.
59. Noda K, Sasaki H, Yamamoto K, Yamamoto T, Nishimura R, Sugiyama T, et al. Phase II trial of topotecan for cervical cancer of the uterus. Proc Am Soc Clin Oncol. 1996;15:280.
60. Abu-Rustum NR, Lee S, Massad LS. Topotecan for recurrent cervical cancer after platinum-based therapy. Int J Gynecol Cancer. 2000;10(4):285–8.
61. Nascimento de Oliveira L, Guerra Alves FV, Ribeiro Mora PA, Calazan do Carmo C, Nogueira-Rodrigues A, Ingles Garces AH, et al. Topotecan use for second-line treatment in patients with recurrent or metastatic cervical cancer at Brazilian National Cancer Institute (INCA). JCT. 2013;4(6):1095–9.
62. Muggia FM, Blessing JA, Method M, Miller DS, Johnson GA, Lee RB, Menzin A, Gynecologic Oncology Group study. Evaluation of vinorelbine in persistent or recurrent squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. Gynecol Oncol. 2004;92(2):639–43.
63. Miller DS, Blessing JA, Bodurka DC, Bonebrake AJ, Schorge JO, Gynecologic Oncology Group. Evaluation of pemetrexed (Alimta, LY231514) as second line chemotherapy in persistent or recurrent carcinoma of the cervix: a phase II study of the Gynecologic Oncology Group. Gynecol Oncol. 2008;110(1):65–70.
64. Thigpen T, Shingleton H, Homesley H, Lagasse L, Blessing J. Cis-platinum in treatment of advanced or recurrent squamous cell carcinoma of the cervix: a phase II study of the Gynecologic Oncology Group. Cancer. 1981;48(4):899–903.

65. Rose PG, Blessing JA, Lele S, Abulafia O. Evaluation of pegylated liposomal doxorubicin (Doxil) as second-line chemotherapy of squamous cell carcinoma of the cervix: a phase II study of the Gynecologic Oncology Group. Gynecol Oncol. 2006;102(2):210–3.
66. Rose PG, Blessing JA, Arseneau J. Phase II evaluation of altretamine for advanced or recurrent squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. Gynecol Oncol. 1996;62(1):100–2.
67. Rose PG, Blessing JA, Van Le L, Waggoner S. Prolonged oral etoposide in recurrent or advanced squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. Gynecol Oncol. 1998;70(2):263–6.
68. Curtin JP, Blessing JA, Webster KD, Rose PG, Mayer AR, Fowler WC Jr, Malfetano JH, Alvarez RD. Paclitaxel, an active agent in nonsquamous carcinomas of the uterine cervix: a Gynecologic Oncology Group study. J Clin Oncol. 2001;19(5):1275–8.
69. Vermorken JB, Tumolo S, Roozendaal KJ, Guastalla JP, Splinter TA, Renard J. 5-aza-2'-deoxycytidine in advanced or recurrent cancer of the uterine cervix. Eur J Cancer. 1991;27(2):216–7.
70. van der Burg ME, Monfardini S, Guastalla JP, de Oliveira C, Renard J, Vermorken JB. Phase II study of weekly 4'-epidoxorubicin in patients with metastatic squamous cell cancer of the cervix: an EORTC Gynaecological Cancer Cooperative Group study. Eur J Cancer. 1992;29A(1):147–8.
71. ClinicalTrials.gov. Phase III study of S-1 + cisplatin vs cisplatin in cervical cancer. NCT00770874.
72. McLachlan J, Boussios S, Okines A, Glaessgen D, Bodlar S, Kalaitzaki R, Taylor A, Lalondrelle S, Gore M, Kaye S, Banerjee S. The impact of systemic therapy beyond first-line treatment for advanced cervical cancer. Clin Oncol (R Coll Radiol). 2017;29(3):153–60.
73. Manders DB, Kehoe SM, Miller DS, Lea JS, Richardson DL. Third-line salvage chemotherapy for recurrent carcinoma of the cervix is associated with minimal response rate and high toxicity. Am J Clin Oncol. 2017; [Epub ahead of print].
74. Tewari KS, Monk BJ. The rationale for the use of non-platinum chemotherapy doublets for metastatic and recurrent cervical carcinoma. Clin Adv Hematol Oncol. 2010;8(2):108–15.
75. Tang X, Zhang Q, Nishitani J, Brown J, Shi S, Le AD. Overexpression of human papillomavirus type 16 oncoproteins enhances hypoxia-inducible factor 1 alpha protein accumulation and vascular endothelial growth factor expression in human cervical carcinoma cells. Clin Cancer Res. 2007;13(9):2568–76.
76. Look KY, Blessing JA, Gallup DG, Lentz SS. A phase II trial of 5-fluorouracil and high-dose leucovorin in patients with recurrent squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. Am J Clin Oncol. 1996;19(5):439–41.
77. Look KY, Blessing JA, Nelson BE, Johnson GA, Fowler WC Jr, Reid GC. A phase II trial of isotretinoin and alpha interferon in patients with recurrent squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. Am J Clin Oncol. 1998;21(6):591–4.
78. Schefter T, Winter K, Kwon JS, Stuhr K, Balaraj K, Yaremko BP, Small W Jr, Sause W, Gaffney D, Radiation Therapy Oncology Group (RTOG). RTOG 0417: efficacy of bevacizumab in combination with definitive radiation therapy and cisplatin chemotherapy in untreated patients with locally advanced cervical carcinoma. Int J Radiat Oncol Biol Phys. 2014;88:101–5.
79. Zighelboim I, Wright JD, Gao F, Case AS, Massad LS, Mutch DG, Powell MA, Thaker PH, Eisenhauer EL, Cohn DE, Valea FA, Alvarez Secord A, Lippmann LT, Dehdashti F, Rader JS. Multicenter phase II trial of topotecan, cisplatin and bevacizumab for recurrent or persistent cervical cancer. Gynecol Oncol. 2013;130:64–8.
80. Bellone S, Frera G, Landolfi G, Romani C, Bandiera E, Tognon G, Roman JJ, Burnett AF, Pecorelli S, Santin AD. Overexpression of epidermal growth factor type-1 receptor (EGFR1) in cervical cancer: implications for Cetuximab-mediated therapy in recurrent/metastatic disease. Gynecol Oncol. 2007;106(3):513–20.

81. Pérez-Regadera J, Sánchez-Muñoz A, De-la-Cruz J, Ballestín C, Lora D, García-Martín R, Mendiola C, Alonso L, Alba E, Lanzós E. Negative prognostic impact of the coexpression of epidermal growth factor receptor and c-erbB-2 in locally advanced cervical cancer. Oncology. 2009;76(2):133–41.
82. Farley J, Sill MW, Birrer M, Walker J, Schilder RJ, Thigpen JT, Coleman RL, Miller BE, Rose PG, Lankes HA. Phase II study of cisplatin plus cetuximab in advanced, recurrent, and previously treated cancers of the cervix and evaluation of epidermal growth factor receptor immunohistochemical expression: a Gynecologic Oncology Group study. Gynecol Oncol. 2011;121(2):303–8.
83. Kurtz JE, Hardy-Bessard AC, Deslandres M, Lavau-Denes S, Largillier R, Roemer-Becuwe C, Weber B, Guillemet C, Paraiso D, Pujade-Lauraine E. Cetuximab, topotecan and cisplatin for the treatment of advanced cervical cancer: a phase II GINECO trial. Gynecol Oncol. 2009;113(1):16–20.
84. Cetina L, Crombet T, Jiménez-Lima R, Zapata S, Ramos M, Avila S, Coronel J, Charco E, Bojalil R, Astudillo H, Bazán B, Dueñas-González A. A pilot study of nimotuzumab plus single agent chemotherapy as second- or third-line treatment or more in patients with recurrent, persistent or metastatic cervical cancer. Cancer Biol Ther. 2015;16(5):684–9.
85. Schilder RJ, Sill MW, Lee YC, Mannel R. A phase II trial of erlotinib in recurrent squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. Int J Gynecol Cancer. 2009;19(5):929–33.
86. Goncalves A, Fabbro M, Lhommé C, Gladieff L, Extra JM, Floquet A, Chaigneau L, Carrasco AT, Viens P. A phase II trial to evaluate gefitinib as second- or third-line treatment in patients with recurring locoregionally advanced or metastatic cervical cancer. Gynecol Oncol. 2008;108(1):42–6.
87. Candelaria M, Arias-Bonfill D, Chávez-Blanco A, Chanona J, Cantú D, Pérez C, Dueñas-González A. Lack in efficacy for imatinib mesylate as second-line treatment of recurrent or metastatic cervical cancer expressing platelet-derived growth factor receptor alpha. Int J Gynecol Cancer. 2009;19(9):1632–7.
88. Monk BJ, Mas Lopez L, Zarba JJ, Oaknin A, Tarpin C, Termrungruanglert W, Alber JA, Ding J, Stutts MW, Pandite LN. Phase II, open-label study of pazopanib or lapatinib monotherapy compared with pazopanib plus lapatinib combination therapy in patients with advanced and recurrent cervical cancer. J Clin Oncol. 2010;28(22):3562–9.
89. Tinker AV, Ellard S, Welch S, Moens F, Allo G, Tsao MS, Squire J, Tu D, Eisenhauer EA, MacKay H. Phase II study of temsirolimus (CCI-779) in women with recurrent, unresectable, locally advanced or metastatic carcinoma of the cervix. A trial of the NCIC Clinical Trials Group (NCIC CTG IND 199). Gynecol Oncol. 2013;130(2):269–74.
90. Stevanović S, Draper LM, Langhan MM, Campbell TE, Kwong ML, Wunderlich JR, Dudley ME, Yang JC, Sherry RM, Kammula US, Restifo NP, Rosenberg SA, Hinrichs CS. Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells. J Clin Oncol. 2015;33(14):1543–50.
91. Petit RG, Mehta A, Jain M, Gupta S, Nagarkar R, Kumar V, Premkumar S, Neve R, John S, Basu P. ADXS11-001 immunotherapy targeting HPV-E7: final results from a phase 2 study in Indian women with recurrent cervical cancer. J Immunother Cancer. 2014;2(Suppl 3):P92.
92. Frenel J, Le Tourneau C, O'Neil B, Ott PA, Piha-Paul SA, Gomez-Roca CA, Van Brummelen E, Rugo HS, Thomas S, Saraf S, Chen M, Vargaet A. Pembrolizumab in patients with advanced cervical squamous cell cancer: preliminary results from the phase Ib KEYNOTE-028 study. J Clin Oncol. 2016;34(suppl). abstract 5515.
93. Monk BJ, Sill MW, Burger RA, Gray HJ, Buekers TE, Roman LD. Phase II trial of bevacizumab in the treatment of persistent or recurrent squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. J Clin Oncol. 2009;27(7):1069–74.
94. Mackay HJ, Tinker A, Winquist E, Thomas G, Swenerton K, Oza A, Sederias J, Ivy P, Eisenhauer EA. A phase II study of sunitinib in patients with locally advanced or metastatic cervical carcinoma: NCIC CTG trial IND.184. Gynecol Oncol. 2010;116(2):163–7.

95. Santin AD, Sill MW, McMeekin DS, Leitao MM Jr, Brown J, Sutton GP, Van Le L, Griffin P, Boardman CH. Phase II trial of cetuximab in the treatment of persistent or recurrent squamous or non-squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. Gynecol Oncol. 2011;122(3):495–500.
96. Hertlein L, Lenhard M, Kirschenhofer A, Kahlert S, Mayr D, Burges A, Friese K. Cetuximab monotherapy in advanced cervical cancer: a retrospective study with five patients. Arch Gynecol Obstet. 2011;283(1):109–13.
97. Lheureux S, Butler M, Clarke B, et al. A phase I/II study of ipilimumab in women with metastatic or recurrent cervical carcinoma: a study of the Princess Margaret and Chicago N01 Consortia. J Clin Oncol. 2015;33(suppl). abstract 3061.
98. Burotto M, Edgerly M, Poruchynsky M, Velarde M, Wilkerson J, Kotz H, Bates S, Balasubramaniam S, Fojo T. Phase II clinical trial of Ixabepilone in metastatic cervical carcinoma. Oncologist. 2015;20(7):725–6.
99. MITO (Multicentre Italian Trials in Ovarian cancer): CERV 2 trial "A randomized phase II study of carboplatin and paclitaxel +/- cetuximab, in advanced and/or recurrent cervical cancer. 2015 ASCO Annual Meeting Abstracts Meeting Library; 2016. Available from http://meetinglibrary.asco.org/content/151507-156
100. Longatto-Filho A, Pinheiro C, Martinho O, Moreira MA, Ribeiro LF, Queiroz GS, Schmitt FC, Baltazar F, Reis RM. Molecular characterization of EGFR, PDGFRA and VEGFR2 in cervical adenosquamous carcinoma. BMC Cancer. 2009;9:212.
101. Wang HL, Lu DW. Overexpression of c-kit protein is an infrequent event in small cell carcinomas of the uterine cervix. Mod Pathol. 2004;17(6):732–8.
102. Zhang XY, Zhang HY, Zhang PN, Lu X, Sun H. Elevated phosphatidylinositol 3-kinase activation and its clinicopathological significance in cervical cancer. Eur J Obstet Gynecol Reprod Biol. 2008;139(2):237–44.
103. Molinolo AA, Marsh C, El Dinali M, Gangane N, Jennison K, Hewitt S, Patel V, Seiwert TY, Gutkind JS. mTOR as a molecular target in HPV-associated oral and cervical squamous carcinomas. Clin Cancer Res. 2012;18(9):2558–68.
104. Feng W, Duan X, Liu J, Xiao J, Brown RE. Morphoproteomic evidence of constitutively activated and overexpressed mTOR pathway in cervical squamous carcinoma and high grade squamous intraepithelial lesions. Int J Clin Exp Pathol. 2009;2(3):249–60.
105. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, Scott CL, Meier W, Shapira-Frommer R, Safra T, Matei D, Fielding A, Spencer S, Dougherty B, Orr M, Hodgson D, Barrett JC, Matulonis U. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol. 2014;15(8):852–61.
106. Kunos C, Deng W, Dawson D, Lea JS, Zanotti KM, Gray HJ, Bender DP, Guaglianone PP, Carter JS, Moore KN. A phase I-II evaluation of veliparib (NSC #737664), topotecan, and filgrastim or pegfilgrastim in the treatment of persistent or recurrent carcinoma of the uterine cervix: an NRG Oncology/Gynecologic Oncology Group study. Int J Gynecol Cancer. 2015;25(3):484–92.
107. A limited access phase I trial of paclitaxel, cisplatin and ABT-888 in the treatment of advanced, persistent, or recurrent carcinoma of the cervix: an NRG/GOG study. 2015 ASCO Annual Meeting Abstracts Meeting Library; 2015. Available from: http://meetinglibrary.asco.org/content/148460-156
108. Lee SJ, Yang A, Wu TC, Hung CF. Immunotherapy for human papillomavirus- associated disease and cervical cancer: review of clinical and translational research. J Gynecol Oncol. 2016;27:e51.
109. Mohanty S, Huang J, Basu A. Enhancement of cisplatin sensitivity of cisplatin-resistant human cervical carcinoma cells by bryostatin 1. Clin Cancer Res. 2005;11(18):6730–7.
110. Yang W, Song Y, Lu YL, Sun JZ, Wang HW. Increased expression of programmed death (PD)-1 and its ligand PD-L1 correlates with impaired cell-mediated immunity in high-risk human papillomavirus-related cervical intraepithelial neoplasia. Immunology. 2013;139(4):513–22.

111. Chemoradiation therapy and Ipilimumab in treating patients with locally advanced cervical cancer. ClinicalTrials.gov website. NCT01711515.
112. Nivolumab in Treating patients with persistent, recurrent, or metastatic cervical cancer. ClinicalTrials.gov website. NCT02257528.
113. Falchook GS, Leidner R, Stankevich E, Piening B, Bifulco C, Lowy I, Fury MG. Responses of metastatic basal cell and cutaneous squamous cell carcinomas to anti- PD1 monoclonal antibody REGN2810. J Immunother Cancer. 2016;4:70.
114. Su X, Chen WJ, Xiao SW, Li XF, Xu G, Pan JJ, Zhang SW. Effect and safety of recombinant Adenovirus-p53 transfer combined with radiotherapy on long-term survival of locally advanced cervical cancer. Hum Gene Ther. 2016;27(12):1008–14.
115. Bisht KS, Bradbury CM, Mattson D, Kaushal A, Sowers A, Markovina S, Ortiz KL, Sieck LK, Isaacs JS, Brechbiel MW, Mitchell JB, Neckers LM, Gius D. Geldanamycin and 17-allylamino-17-demethoxygeldanamycin potentiate the in vitro and in vivo radiation response of cervical tumor cells via the heat shock protein 90-mediated intracellular signaling and cytotoxicity. Cancer Res. 2003;63:8984–95.
116. A study of MK1775 in combination with topotecan/cisplatin in patients with cervical cancer (1775–008). ClinicalTrials.gov website. NCT01076400.
117. Wang Y, Decker SJ, Sebolt-Leopold J. Knockdown of Chk1, Wee1 and Myt1 by RNA interference abrogates G2 checkpoint and induces apoptosis. Cancer Biol Ther. 2004;3:305–13.
118. Pohlmann P, DiLeone LP, Cancella AI, Caldas AP, Dal Lago L, Campos O Jr, Monego E, Rivoire W, Schwartsmann G. Phase II trial of cisplatin plus decitabine, a new DNA hypomethylating agent, in patients with advanced squamous cell carcinoma of the cervix. Am J Clin Oncol. 2002;25:496–501.
119. Feldman R, Gatalica Z, Reddy S, Tewari K. Paving the road to personalized medicine in cervical cancer: Theranostic biomarker evaluation in a 592-specimen library. Gynecol Oncol. 2015;137(Suppl 1):141.

Chemotherapy for Cervical Cancer

12

Romelie Rieu and Gemma Eminowicz

Epidemiology

Worldwide, cervical cancer is the fourth most common cancer in women. Global mortality varies significantly depending on a country's GDP and the availability of healthcare in particular access to screening, radiotherapy (RT) and chemotherapy [1].

Human papilloma virus (HPV) infection is the major risk factor for developing cervical cancer, in particular subtypes 16 and 18. HPV oncogenes E6 and E7 inhibit DNA damage response mechanisms and lead to chromosomal instability, chromosomal mutations and hence transformation of dysplastic cells into invasive cancers [2]. Viral mRNA also modulates angiogenic factors and the host immune response, both key for progression of invasive cancer and response to systemic treatments such as chemotherapy, immunotherapy or targeted agents.

The precancerous, dysplastic phase and early invasive disease can be detected by cytological screening with the Papanicolaou (Pap) smear test. Its introduction has improved survival rates by reducing the incidence of invasive disease and reducing the stage of disease when invasive cancer is diagnosed [3]. Compliance with screening is not universal and its global availability varies. Hence many women still present with advanced or metastatic disease. HPV vaccination is an alternative method of exploiting this known biology and has been introduced as primary prevention for cervical cancer in the UK in recent years.

R. Rieu
Department of Clinical Oncology/Radiotherapy, Charing Cross Hospital, Imperial College Healthcare NHS Trust, London, UK
e-mail: romelie.rieu@nhs.net

G. Eminowicz (✉)
Department of Clinical Oncology/Radiotherapy, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, UK
e-mail: gemmaeminowicz@nhs.net

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_12

Histology

Squamous cell cancer, a RT- and chemotherapy-sensitive cell type, is the most common histological subtype of cervical cancer, accounting for 70–80%. Other subtypes include adenocarcinoma (10–15%), small-cell neuroendocrine (2%) and other rarer subtypes [4]. Adenocarcinomas and squamous cell cancers are treated with the same chemotherapy regimens, but certain subtypes, such as small-cell, warrant a different approach as discussed later.

Staging

Staging is clinical, based on the International Federation of Gynaecology and Obstetrics (FIGO) system. Locally confined tumours, FIGO stages IA and IBI, are treated with surgery alone, and there is very little role for chemotherapy. Locally advanced cancers, FIGO stages IB to IVA, are treated curatively with chemoradiation. FIGO stage IVB indicates distant metastatic disease, and the mainstay of treatment is palliative chemotherapy.

Proven Roles of Chemotherapy

Localised Disease: Chemotherapy with Radiotherapy

For FIGO stages IB to IVA, radical RT involving external beam radiotherapy (EBRT) and brachytherapy (BT) has been shown to confer equivalent disease-free survival at 5 years to surgery, with less morbidity [5]. However, pelvic RT by itself fails to control progression of locally advanced disease in 40–60% of patients, and two-thirds of patients who recur do so within the RT field [6, 7]. Whilst the combination of RT and surgery has been shown to improve disease-free survival, it has not been confirmed to improve overall survival (OS) and is associated with increased morbidity [8, 9]. Where patient fitness allows, the addition of chemotherapy to radical RT can sensitise tumours to radiation and eradicate micro-metastases.

Cisplatin as a Radiosensitiser

Concurrent cisplatin reduces and delays local recurrence within the RT field and, to a lesser extent, reduces distant recurrence and progression. It has both a radiosensitising effect and systemic anticancer properties [10].

Cisplatin forms inter- and intra-strand cross-links within DNA and RNA, blocking nucleotide replication and transcription and inducing cell death. Importantly, it is active in both hypoxic and oxygenated cells, unlike RT which requires oxygen and free radical production to maximise DNA damage. The level of cell death achieved when cisplatin is used in combination with RT is more than would be

expected by the sum of the individual modalities. Cisplatin integrates into DNA and RNA in close proximity to a RT-induced single-strand break, impeding DNA repair. This is partly through cisplatin's free electron-scavenging capacity that inhibits sub-lethal damage repair, a process implicated in the recovery of insufficiently irradiated cells [11]. Moreover, ionisation radiation can increase cellular uptake of platinum chemotherapies [12]. It is important to balance the potential benefits of radiosensitisation with the potential increased toxicity experienced as a result of co-administration.

Benefit of Concurrent Cisplatin Chemo-Radiation

Five practice-changing trials investigating the role of cisplatin-based chemotherapy with RT for locally advanced cervical cancers were reported in 1999 (Table 12.1), including the Radiation Therapy Oncology Group (RTOG) 90–01 and the Gynecologic Oncology Group (GOG) 123, GOG 109, GOG 120 and GOG 85 [13–17]. Inclusion criteria, standard treatment arms, chemotherapy doses and the other adjuvant treatments received by patients differed in each study. The RTOG 90–01 compared RT alone to RT with concurrent 5-fluorouracil (5-FU) and cisplatin in patients with stages IIB–IVA disease. GOG 123 compared RT alone to RT with concurrent weekly cisplatin 40 mg/m2 in bulky stage IB disease. GOG 109

Table 12.1 Summary of the five key studies confirming benefit of concurrent chemotherapy with radiotherapy

Trial	Stage	No. of patients	Arms	Survival	Significance
				5-yr DFS	$P < 0.001$
RT 90–01	IIB–IVA	403	RT RT/5-FU/cisplatin	40% 67%	
				3-yr OS	*P = 0.008*
GOG 123	IB	379	RT + surgery RT/cisplatin + surgery	74% 83%	
				Est. 4-yr PFS	$P = 0.003$
GOG 109	IA2–IIA	268	Surgery + RT Surgery + RT/ cisplatin/5-FU	63% 80%	
				2-yr PFS	$P < 0.001$
GOG 120	IIB–IVA	526	RT/cisplatin RT/cisplatin/5-FU/HU RT/HU	67% 64% 47%	
				5-yr PFS	$P = 0.033$
GOG 85	IIB–IVA	368	RT/oral HU RT/5-FU infusion/bolus cisplatin	47% 57%	

RT radiotherapy, *DFS* disease-free survival, *PFS* progression-free survival, *5-FU* 5-fluorouracil, *HU* hydroxyurea

compared radical RT alone to concurrent three-weekly cisplatin 70 mg/m2 and 5-FU for patients with high-risk post-operative clinical stages IA2–IIA cervical cancer. All three demonstrated statistically significant and clinically important OS advantages with concurrent chemotherapy over RT alone and a relative risk reduction of cancer recurrence of approximately 50%. The GOG 120 study compared three concurrent chemotherapy regimens: (i) cisplatin alone; (ii) cisplatin, 5-FU and hydroxyurea; and (iii) hydroxyurea alone. The cisplatin-containing regimens significantly decreased disease progression compared to the group receiving hydroxyurea alone ($p < 0.001$ for both comparisons), cisplatin alone (relative risk of disease progression 0.57; 95% confidence interval, 0.42–0.78), and cisplatin, fluorouracil and hydroxyurea (relative risk of disease progression 0.55; 95% confidence interval, 0.40–0.75). The GOG 85 compared concurrent 5-FU plus cisplatin versus single-agent hydroxyurea alongside radical RT in stages IIB–IVA cervical cancer with negative para-aortic lymph nodes. Again, combination chemotherapy of cisplatin and 5-FU was associated with significant improvement in progression-free survival (PFS) and OS and significantly less grade 3 or 4 haematologic toxicity than patients receiving adjuvant hydroxyurea.

Taken together, these studies established the role of concurrent cisplatin-containing chemotherapy alongside radical RT for cervical cancer and triggered its recommendation by the National Cancer Institute (NCI). This practice has been widely adopted in the UK. The most commonly used regimen is five to six weekly cycles of 40 mg/m2 (max 70 mg) of concurrent cisplatin [18].

In 2011, a meta-analysis based on the individual patient data from 15 trials (including the 5 trials discussed above) across 11 countries confirmed a 6% improvement in 5-year OS from 60% to 66%, HR 0.81, and an 8% improvement in DFS from 50% to 58% with chemotherapy [19]. After excluding two trials in which additional adjuvant chemotherapy was administered, similar benefits in OS and other outcomes were seen.

Chemotherapy inferred similar and significant absolute benefit (6–9%) in terms of 5-year loco-regional disease-free survival, time to loco-regional recurrence/progression and metastases-free survival. The smaller improvement in metastases-free interval at 5 years, defined as time from randomisation to first metastasis (4%, $p = 0.037$), suggests the benefit from chemotherapy is primarily from improving local control within the RT field, with only a minor role in treating pre-existing micro-metastatic deposits.

Whilst the benefits were seen across all stages regardless of age, histology and grade, they appeared to be lower in patients with more advanced disease. For stages IB–IIA, there was a 10% 5-year survival benefit, compared with a 7% benefit for stage IIB and 3% for stages III–IVA. There was no effect of chemotherapy type (platinum-based or non-platinum-based), RT dose, total RT duration or dose intensity of cisplatin (when included). Whilst similar benefits were seen with platinum-based and non-platinum-based chemotherapy regimens, the analysis did not include any head-to-head comparisons that support this.

Acute toxicities significantly increase when adding single-agent or combination chemotherapy to RT, including haematologic (two- to tenfold increase),

gastrointestinal, genitourinary and skin toxicities. However, very few serious events are noted. Late toxicity analysis was not possible due to incomplete or missing information from the primary studies, and only one study included a quality of life assessment.

Combination Chemo-Radiation Treatment: Gemcitabine and Cisplatin

Gemcitabine, an antimetabolite chemotherapy that inhibits DNA repair and production, acts synergistically with RT and cisplatin. In 2011, Dueñas-González et al. randomised approximately 500 patients with FIGO IIB–IVA cervical cancer to EBRT and concurrent cisplatin followed by BT or EBRT with concurrent cisplatin and gemcitabine chemotherapy followed by BT and then two adjuvant cycles of cisplatin and gemcitabine [20]. A 10.6% 3-year PFS improvement was reported, although the contributions of adjuvant versus concurrent chemotherapy cannot be elucidated given the lack of appropriate controls and that adjuvant chemotherapy is not a standard practice.

The median duration of RT was longer in the chemotherapy arm due to delays secondary to toxicity. Extending overall treatment time compromises RT survival benefit [21, 22]. Furthermore, only 86% of patients due to receive adjuvant treatment received their first cycle because of chemo-RT toxicity, and toxicity information was only collected for 1-year post-treatment. These side effects were reported as clinically manageable and similar to those reported in previous studies [23, 24]. However, it conflicts with other studies concluding that gemcitabine and cisplatin combination chemo-RT is associated with an unacceptable increase in dose-limiting toxicities including myelosuppression, diarrhoea, nausea and vomiting [25, 26]. The discrepancy may be explained by the differing sequence of chemotherapy administration; studies experiencing more toxicity administered gemcitabine before cisplatin, potentially pre-blocking the nuclear excision repair proteins and maximising the cisplatin effect, but also maximising toxicity.

Combination Chemo-Radiation Treatment: 5-Fluorouracil and Cisplatin

Cisplatin and 5-FU combination chemotherapy given concurrently with RT was a commonly used regimen after GOG studies (e.g. GOG 120 and GOG 109) demonstrated an associated OS benefit over RT alone or non-platinum-based chemotherapy such as hydroxyurea.

A Korean research group performed the first head-to-head comparison of cisplatin alone versus combination 5-FU and cisplatin with radical RT for locally advanced cervical cancer [27]. Cisplatin alone was given at 30 mg/m2 (rather than 40 mg/m2). Compared to other studies, this lower dose was associated with fewer severe acute toxicities, and a larger proportion of patients completed the full six cycles of

treatment (71%). Within the study, both cisplatin alone and cisplatin plus 5-FU regimens had similar 4-year survival rates. The 5-FU cohort were admitted to deliver long transfusions and suffered significantly more grade 3 and grade 4 adverse events (43% versus 26%, $p = 0.037$). This supports the use of single-agent cisplatin chemotherapy at a dose of at least 30 mg/m2.

Metastatic and Recurrent Disease: First-Line Treatment

Approximately 5% of new cervical cancer diagnoses are metastatic, and between 15% and 61% of early stage patients will develop metastatic disease [28]. Cervical cancer metastasises by lymphatic and haematogenous spread, most commonly to para-aortic lymph nodes and then the lungs, liver and bones [29–31]. Relapse is most common within 2 years of completing primary treatment. Five-year survival is between 6% and 77%, depending on recurrence location and treatment options available [32]. Treatment decisions should be made within a multidisciplinary team.

Locally recurrent disease, especially if the patient is RT naïve or fit for complete surgical exenteration, may be treated curatively. However, if distant metastatic disease is present, the patient has been heavily pretreated or is unfit; palliative chemotherapy is the mainstay of treatment. Many different regimens are used, as documented in Table 12.2.

Table 12.2 Example of chemotherapy regimens used for metastatic or recurrent cervical cancer

First-line recurrent or metastatic disease	
Carboplatin + paclitaxel	Carboplatin 5 AUC + paclitaxel 175 mg/m^2 every 3 weeks
Bevacizumab	Bevacizumab 15 mg/kg every 3 weeks in combination with carboplatin + paclitaxel first-line recurrent/metastatic disease
Single agents	Cisplatin 75 mg/m^2 every 3 weeks Carboplatin 5 AUC every 3 weeks Paclitaxel 250 mg/m^2 every 3 weeks
Second-line treatments	
Carboplatin + paclitaxel	Carboplatin 5 AUC + paclitaxel 175 mg/m^2 every 3 weeks (if long disease-free interval with good response to first-line treatment) Carboplatin 5 AUC + paclitaxel 80 mg/m^2 D1, 8, 15 every 4 weeks Carboplatin 2 AUC weekly/paclitaxel 70 mg/m^2 weekly continuous
Single agents	Paclitaxel 75–80 mg/m^2 weekly (either continuous or D1 + D8 + D15 every 4 weeks) Cisplatin 75 mg/m^2 every 3 weeks Carboplatin 5 AUC every 3 weeks Carboplatin 2 AUC weekly
Cisplatin-based regimens	Cisplatin 50 mg/m^2 + topotecan 0.75 mg/m^2 every 3 weeks Cisplatin 50 mg/m^2 + doxorubicin 60 mg/m^2 every 3 weeks
Docetaxel-based regimens	Docetaxel 75 mg/m^2 + gemcitabine 1000 mg/m^2 every 3 weeks

First-Line Chemotherapy

Platinum-Based Chemotherapy

Early studies demonstrated efficacy of single-agent cisplatin in locally advanced, metastatic and recurrent cervical cancer [33]. However, cisplatin is highly nephrotoxic, often complicated by disease-related renal dysfunction (ureteric obstruction, ascites or dehydration), and highly emetogenic, which can be dose limiting and contribute to dehydration and renal dysfunction. Electrolyte disturbance, neuropathy and ototoxicity are other dose-limiting adverse effects.

Given the palliative intent of chemotherapy in the metastatic setting, therapeutically equivalent options with more favourable toxicity profiles are desirable. Carboplatin has the same mechanism of action but less nephrotoxicity, neurotoxicity and is less emetogenic. Dosage is calculated according to renal filtration, and infusion time is quicker with less rehydration necessary.

Carboplatin dose-limiting effects include myelosuppression, in particular thrombocytopenia, often increased in patients with prior pelvic RT. However, when paclitaxel is administered before carboplatin, it has a "platelet-sparing effect", reducing thrombocytopenia [34]. A phase III study of 250 metastatic cervical cancer patients, the JCOG 0505, reported that paclitaxel with carboplatin was non-inferior to cisplatin and paclitaxel with less toxicity and time spent in hospital [35].

A retrospective systematic review comparing cisplatin and carboplatin plus paclitaxel-based chemotherapy for recurrent or metastatic cervical cancer evaluated 1181 patients within 17 studies [36]. No significant difference in response rate or OS between carboplatin and cisplatin was seen. In a subgroup analysis, prior platinum exposure with RT reduced response rates to subsequent treatment, again with no difference between cisplatin and carboplatin treatment arms. Carboplatin is therefore considered an alternative to cisplatin and is substituted into cisplatin-based regimens.

Combination Chemotherapy

Many studies have investigated combination regimens aiming to increase response rates and survival. The addition of paclitaxel to carboplatin increased the objective response rate (from 16% to 36%) and median PFS (2.8–4.8 months) [37]. However, combination treatment was associated with more neutropenia and leucopenia. The GOG 204 trial compared four regimens in first-line treatment for advanced (stage IVB), recurrent or persistent cervical cancer. Just over 100 patients received each arm, standard paclitaxel + cisplatin (PC), vinorelbine + cisplatin, gemcitabine + cisplatin or topotecan + cisplatin [38]. No significant OS difference was seen, but the median OS was longest for patients receiving PC at 12.87 months. Less than 30% of patients responded to any treatment, defined by RECIST criteria. PC was associated with significantly more alopecia than any other regimen and significantly more neutropenia and leucopenia than gemcitabine + cisplatin, not affecting quality of life. Performance status, but not age, was a significant prognostic factor. Economic analysis of cisplatin versus cisplatin doublet showed PC as the most cost-effective regimen [39].

The increased treatment-associated toxicity, disappointing response rate and short OS with combination chemotherapy fuel the need for improved predictive markers and new targeted and biological treatments.

In 2010 a retrospective analysis of tumour response data in GOG protocol-treated patients defined five factors associated with tumour response rate: race (black versus non-black), performance status, site of disease (pelvic versus non-pelvic), prior chemo-RT treatment with cisplatin (versus no chemotherapy) and interval between diagnosis and first recurrence (recurrence within 1 year versus longer) [40]. The prognostic model using these factors identified 16% as high risk and unlikely to respond to treatment, implying they could be spared the toxicity of chemotherapy. These predictive factors are not fully consistent with other studies and therefore need further validation.

Anti-angiogenic Treatment

Vascular endothelial growth factor (VEGF) is a key mediator of tumour angiogenesis involved in mitogenesis, endothelial cell survival and haematopoiesis [41]. Levels of VEGF are regulated in healthy tissues by hypoxia-inducible factor 1a (HIF1a). Both VEGF and HIF1a are upregulated in invasive cervical cancer [42]. Bevacizumab is a fully humanised monoclonal antibody against VEGF.

Therefore, the landmark phase III GOG 240 trial investigated adding bevacizumab to either standard PC or topotecan and paclitaxel (TP) in first-line metastatic, persistent or recurrent cervical carcinoma. Bevacizumab, compared to both chemotherapy combinations, improved OS from 13.3 to 17 months (HR 0.71; 98% confidence interval [CI], 0.54–0.95; $p = 0.004$) [43]. Even among patients with prior platinum exposure, no significant difference was found between the two chemotherapy combinations. The OS is much greater than the previously reported median survival of between 8 and 14 months and reflects strict selection criteria for patients (performance score of 0 or 1) and medical optimisation of patients, as well as the additional benefit from bevacizumab.

Response rate also increased significantly with bevacizumab (48% vs 36%; 95% CI, 1.08 to 1.68, $P = 0.008$). However, bevacizumab was associated with significantly more grade 2+ hypertension (25% vs 2%), grade 3+ thromboembolic events (8% vs 1%) and grade 3+ gastrointestinal fistulas (3% vs 0%). It was also associated with reduced neurotoxic symptoms, possibly due to secondary gain from increased tumour shrinkage, better health or more activity in patients receiving bevacizumab or bevacizumab-induced myalgia modulating the perceived neuropathy via the gate theory of pain. Objective neuropathy assessment is needed to elucidate this further. Importantly, the toxicity profile did not adversely affect the patients' health-related quality of life [44].

The 3.7-month median survival improvement with bevacizumab is clinically meaningful. The success of bevacizumab may also represent a paradigm shift towards biological and targeted agents to improve survival in this relatively chemo-refractory disease. However, when considering treatments which offer modest survival benefits and potential additional toxicities, strict patient selection and medical optimisation are critical.

Second-Line Treatments

There is no standard second-line chemotherapy in recurrent and metastatic cervical cancer. Once the disease has progressed after first-line treatment or if patients are not candidates for combination chemotherapy, the prognosis is poor. No treatment confers a proven OS benefit. Well-designed clinical trials are required to explore new treatment options and elucidate best practice. Entry into such trials should be encouraged.

Given their limited activity, tolerability of treatments is critical. For this reason, single-agent or dose-reduced chemotherapies are commonly used (Table 12.2). A recent retrospective single-centre review of second-line therapy in 53 patients revealed weekly paclitaxel to be the most commonly used treatment (28.3%); other options included carboplatin-based chemotherapy, targeted agent monotherapy within a clinical trial, docetaxel-based chemotherapy, topotecan and gemcitabine [45]. The objective response rate was <15%. The clinician's choice may be influenced by patient's fitness, symptoms, prior therapies and residual toxicity.

(Small-Cell) Neuroendocrine Cancer (SCNEC)

Neuroendocrine tumours are a rare histological subtype accounting for approximately 2% of cervical cancer. Their rarity means there are no prospective trials or consensus guidelines to direct management. Analysis of small retrospective studies is challenging because standard RT and chemotherapy protocols vary, the decision-making process for adjuvant treatment strategies is not explored and the effect of bias and confounding factors is large. This summary focuses on small-cell neuroendocrine cancers (SCNECs), the most common subtype. Less common variants include large cell, typical carcinoid and atypical carcinoid tumours.

Histologically SCNEC is indistinguishable from small-cell carcinoma of the lung, with up to 82% demonstrating lympho-vascular space infiltration. The diagnostic pathway and staging are also similar, dividing the tumours into limited and extensive stage disease.

They are generally aggressive, metastasise early and have a poor 5-year survival of 0–30% [46]. Poor prognostic factors include smoking, tumour size, lymph node involvement and pure small-cell histology [47, 48]. A multimodal treatment approach is favoured, with surgery, chemotherapy and RT considered for all patients.

SCNEC Role of Surgery

Radical hysterectomy has been shown to be necessary for long-term survival from SCNEC and is performed either first line or in the adjuvant setting for tumours over 4 cm [49, 50].

SCNEC Chemotherapy

SCNEC is generally chemosensitive and therefore chemotherapy is used in every stage of the disease. A retrospective review in 23 patients, with a median follow-up of 41 months, demonstrated a 68% survival with vincristine, doxorubicin and

cyclophosphamide alternating with cisplatin and etoposide (VAC/PE, $n = 14$) compared with 33% with cisplatin, vinblastine and bleomycin (PVB, $n = 9$) ($P = 0.0078$, log rank test) [51]. The authors therefore recommended VAC- or PE-containing regimens, reflecting similarities with small-cell lung cancer, and proposed that higher-dose intensity adjuvant chemotherapy may confer better survival for early stage, operable SCNEC.

Chemotherapy is thought to reduce recurrence risk in limited stage disease. In a retrospective case series of 11 patients with localised disease, 6 had platinum/etoposide combination chemotherapy. 100% (5 out of 5) who did not receive adjuvant chemotherapy developed distant metastases within 2 years and died within 3 years, whilst only 33% (2 of 6 patients, $P = 0.015$) recurred in the adjuvant chemotherapy group, with a 63% 3-year survival rate ($P = 0.045$) [52].

A subsequent larger retrospective study of 34 patients with stages IIB–IVA reported improved OS with chemotherapy (3-year survival, 17.8% vs 12%, $P = 0.43$) [49]. Cisplatin/etoposide was the most common regimen.

SCNEC Chemo-Radiation Therapy and Future Hopes

Whilst the use of RT for SCNEC is common, the evidence it improves survival is poor. There are ongoing trials evaluating targeted agents such as gefitinib, bevacizumab, temsirolimus, sorafenib and thalidomide in small-cell cancers.

Current Unproven Roles of Chemotherapy

Neoadjuvant Chemotherapy

In theory, neoadjuvant chemotherapy (NACT) given before definitive treatment, either preoperatively in early stage disease or prior to RT in locally advanced disease, reduces tumour burden. This may render inoperable tumours operable, permit fertility-sparing surgical techniques or improve the oxygenation profile allowing maximal radiation-induced cell kill [53]. It may also eliminate distant micrometastatic disease that would not be treated with localised treatment, reducing recurrence [54]. Furthermore, the delay between diagnosis and the start of treatment is often shorter in patients treated with chemotherapy than either surgery or RT, which both require more complex workup and planning. Benefits of NACT depend on the cancer being chemotherapy-sensitive. Monitoring tumour response permits changes in management (for instance, if resistant cells grow through treatment) and can be used as a biological marker to predict outcomes [55]. NACT is not currently a standard practice due to the lack of consistent, good quality evidence demonstrating benefit.

Neoadjuvant Chemotherapy (NACT) Prior to Surgery

Early studies were small, used various doses and schedules and did not control for differing uses of adjuvant RT. Despite this, an early meta-analysis suggested a

significant increase in 5-year survival using NACT preoperatively, hazard ratio of 0.65 and 14% absolute improvement [56].

Two subsequent large randomised control trials (RCTs) investigating whether three cycles of platinum-based NACT improved outcome in locally advanced cervical cancer were slow to recruit and did not find statistical benefit in terms of OS or PFS [57, 58]. The GOG 141 was closed early due to poor accrual and off-protocol use of adjuvant RT.

In 2003, a feasibility study of 43 patients with stages IB2–IIIB cervical cancer treated with three cycles of paclitaxel/cisplatin NACT prior to surgical hysterectomy and adjuvant chemo-RT achieved clinical responses in 95% of patients [59]. Acceptable toxicity and 90% of patients completing all scheduled treatment were reported. However, in 2009 the Italian Collaborative Study Group published a similar study demonstrating optimal response rates in 42–48% and significant haematological toxicity [60].

The 2010 Cochrane meta-analysis of NACT before surgery included five studies (n = 604 women) and demonstrated a PFS improvement (HR 0.76, P = 0.01) and a trend towards OS improvement. A further meta-analysis of five RCTs and four observation studies (n = 1784 women) again showed no OS benefit [61]. The limitations of these studies include the trial heterogeneity, inconsistent use of adjuvant RT and inclusion of observational studies.

Overall, the current evidence is conflicting. Despite this, in parts of the world where access to RT is limited, NACT prior to surgery is used for locally advanced cervical cancer. Thus, the results of the European Organisation for Research and Treatment of Cancer, EORTC, 55994 phase III study investigating this are eagerly awaited.

Neoadjuvant Chemotherapy Before Radiation/Chemo-Radiation

A 2003 meta-analysis evaluated NACT before RT versus RT alone. However, as previously discussed, chemo-RT is the treatment of choice for locally advanced cervical cancer. The "standard" comparison of RT alone is therefore out-of-date limiting validity of results [57]. That said, 21 RCTs were included, and a 5-year survival improvement with high-dose intensity, accelerated platinum-based NACT was reported.

More recently, a multicentre phase II trial of 46 women, CXII, investigated the feasibility of dose-dense weekly carboplatin (AUC2) and paclitaxel (80 mg/m2) for 6 weeks prior to conventional chemo-RT, for stages IIB–IVA cervical cancer [62]. Complete or partial response was achieved in 70% of patients at the end of NACT and 85% at 12 weeks post-chemo-RT. There was an acceptable toxicity profile; 98% completed the subsequent chemo-RT within 50 days and 78% completed at least four cycles of concomitant cisplatin. The most common dose-limiting toxicity was haematological, with 20% developing grade 3 or 4 toxicity during NACT. The 5-year survival was 67% and included three of five patients with positive para-aortic lymph nodes who were alive with no evidence of disease and who would have been deemed high risk at baseline. These results have justified the currently recruiting

international phase III INTERLACE trial investigating the same dose-dense combination NACT prior to radical chemo-RT treatment [63].

Adjuvant Chemotherapy After Chemo-Radiation

The role of adjuvant chemotherapy in cervical cancer is unclear. Theoretically, adjuvant treatment can reduce recurrence risk by eliminating viable malignant cells both within the RT field and distant micro-metastases.

The chemo-RT meta-analysis, CCCMAC 2008, demonstrated a larger OS benefit in patients receiving additional adjuvant chemotherapy following radical chemo-RT. However, this analysis was based on two small studies of differing designs, for which it was not possible to determine the relative contributions of concurrent and adjuvant chemotherapy, and one study investigated non-platinum combination regimens.

Dueñas-Gonzalez et al. showed a significant survival advantage for patients who underwent combination chemo-RT in combination with adjuvant chemotherapy (3-year PFS 74% vs 65%, $P = 0.029$); however the chemo-RT regimen was also changed, and therefore, again, there was no adequate control to confirm the benefit of adjuvant chemotherapy [64]. The latest Cochrane review could not confirm any benefit from adjuvant treatment, and it is still not a standard practice [65].

At the time of writing, a multicentre international randomised GOG and RCOG study, OUTBACK, is currently recruiting [66]. OUTBACK is investigating standard chemo-RT with five cycles of cisplatin followed by BT with or without four adjuvant cycles of three-weekly carboplatin and paclitaxel.

Future Directions for Treatment

Immunotherapy

The exploration and exploitation of immune modulating therapies in cancer treatment is arguably the most revolutionary advance in systemic anticancer treatment in recent times. There are high hopes that ongoing research using immunotherapies in cervical cancer will deliver practice-changing results.

Cancer cells must evade or suppress the immune system [67]. Immunotherapy aims to “reactivate” suppressed immune responses, allowing the host to target cancer antigens. Laurence et al. demonstrated mutational heterogeneity between cancers [68]. Highly mutagenic cancers (e.g. melanoma and lung cancers) are more susceptible to immunotherapy because they have many antigens for the immune system to target. HPV-positive cervical cancers may be more receptive to immunotherapy than HPV-negative cervical cancers because HPV oncogenes E6 and E7 degrade p53 and inactivate pRb, respectively (both of which are key tumour suppressor gene products involved in the DNA damage response), leading to greater chromosomal instability and mutagenicity [69, 70].

Checkpoint inhibitors block inhibitory signals suppressing T cell activation. Nivolumab and ipilimumab are monoclonal antibodies against cell surface proteins PD-1 (programmed cell death protein-1) and CTLA-4 (cytotoxic T lymphocyte-associated protein-4), respectively. Research into their role in cervical cancer management is ongoing. The CheckMate 358 trial is a phase I/II trial of neoadjuvant nivolumab or combination nivolumab and ipilimumab in virus-associated tumours including cervical cancer [71]. The estimated completion date is May 2018. In January 2017 a phase II trial of nivolumab and ipilimumab in patients with rare tumours including cervical adenocarcinoma was opened [72]. The primary outcome measure is overall response rate, with recruitment due to close in August 2020.

Future studies will need to address the uncertainties of immunotherapy including the absence of standardised predictive factors, the unpredictability of side effects, the controversies in tumour response evaluation and the optimal schedules and dosages.

Therapeutic Vaccines

Due to the HPV-driven biology of cervical cancer, therapeutic vaccines have previously been thought to be a potential attractive avenue of research. However, all attempts to produce therapeutic vaccines against tumour antigens, including the oncogenes E6 and E7, have been disappointing.

Summary

Whilst surgery and RT can be definitive treatments for cervical cancer, chemotherapy has a supplementary role in all stages, especially where access to RT is limited. Platinum-based chemotherapy given concurrently with radical RT improves OS in patients with locally advanced cancer and is a standard of care. In the metastatic setting, a benefit in OS has been shown with platinum-based combination chemotherapy including, where appropriate, the monoclonal antibody bevacizumab. Second-line treatment options are less evidence-based, and if patients are fit, referral to specialist units for enrolment into clinical trials is recommended.

Small-cell neuroendocrine cervical cancers represent a rare histological subtype, associated with a poor prognosis. Despite limited evidence, multimodal treatments are common, with etoposide and platinum combination chemotherapy considered in all stages of disease.

Other potential roles of chemotherapy include use in the neoadjuvant and adjuvant setting to reduce recurrence risk and improve survival. Due to a lack of evidence, there are some international randomised trials attempting to address these questions. Neoadjuvant chemotherapy preoperatively in early stage disease and pre-chemo-RT in locally advanced disease are currently under investigation in the EORTC 55994 and INTERLACE trials, respectively. OUTBACK is investigating adjuvant chemotherapy after chemo-RT in locally advanced disease.

Research into the future role of checkpoint inhibitors, vaccines and targeted treatments is also ongoing and represents a potential paradigm shift in the management of cervical cancer.

References

1. Abdel-Wahab M, et al. Global access to radiotherapy in low- and middle-income countries. Clin Oncol (R Coll Radiol). 2017;29(2):99–104.
2. Clere N, et al. The human papillomavirus type 18 E6 oncoprotein induces vascular endothelial growth factor 121 (VEGF121) transcription from the promoter through a p53-independent mechanism. Exp Cell Res. 2007;313:3239–50.
3. Comparetto C, et al. Cervical cancer screening: a never-ending developing program. World J Clin Cases. 2015;3(7):614–24.
4. Colombo N, et al. Cervical cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up ESMO Guidelines Working Group. Ann Oncol. 2012;23(Suppl 7):vii27–32.
5. Landoni F, et al. Randomised study of radical surgery versus radiotherapy for stage Ib-IIa cervical cancer. Lancet. 1997;350(9077):535–40.
6. Perez CA, et al. Radiation therapy alone in the treatment of carcinoma of uterine cervix. I. Analysis of tumor recurrence. Cancer. 1983;51(8):1393–402.
7. Jampolis S, et al. Analysis of sites and causes of failures of irradiation in invasive squamous cell carcinoma of the intact uterine cervix. Radiology. 1975;115:681–5.
8. Rogers L, et al. Radiotherapy and chemoradiation after surgery for early cervical cancer. Cochrane Database Syst Rev. 2012;5:CD007583. https://doi.org/10.1002/14651858.CD007583.pub3.
9. O'Quinn AG, et al. Guidelines for conservative hysterectomy after irradiation. Gynecol Oncol. 1980;9:68–79.
10. Seiwet TY, et al. The concurrent chemoradiation paradigm – general principles. Nat Clin Pract Oncol. 2007;4(2):86–100.
11. Wilson GD, et al. Biologic basis for combining drugs with radiation. Semin Radiat Oncol. 2006;16:2–9.
12. Yang LX, et al. Irradiation enhances cellular uptake of carboplatin. Int J Radiat Oncol Biol Phys. 1995;33(3):641–6.
13. Morris M, et al. Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer. New Engl J Med. 1991;340(15):1137–43.
14. Keys HM, et al. Cisplatin, radiation, and adjuvant hysterectomy compared with radiation and adjuvant hysterectomy for bulky stage IB cervical carcinoma. N Engl J Med. 1999;340(15):1154–61.
15. Peters WA, et al. Concurrent chemotherapy and pelvic radiation therapy compared with pelvic radiation therapy alone as adjuvant therapy after radical surgery in high-risk early-stage cancer of the cervix. J Clin Oncology. 2000;18(8):1606–13.
16. Rose PG, et al. Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. New Engl J Med. 1999;340(15):1144–53.
17. Whitney CW, et al. Randomised comparison of fluorouracil plus cisplatin versus hydroxyurea in stage IIB/IVA in carcinoma of the cervix. J Clin Oncol. 1999;17:1339–48.
18. McCormack M, et al. A national audit of chemo-radiation practice for cervical carcinoma in the United Kingdom. Royal College of Radiologists Annual Scientific Meeting 2001. Clin Oncol. 2001;13:318–9.
19. CCCMAC. Reducing uncertainties about the effects of chemoradiotherapy for cervical cancer: a systematic review and meta-analysis of individual patient data from 18 randomized trials. J Clin Oncol. 2008;26:5802–12.

20. Dueñas-González A, et al. Phase III, open-label, randomized study comparing concurrent gemcitabine plus cisplatin and radiation followed by adjuvant gemcitabine and cisplatin versus concurrent cisplatin and radiation in patients with stage IIB to IVA carcinoma of the cervix. J Clin Oncol. 2011;29:1678.
21. Fyles AW, et al. Prognostic factors in patients with cervix cancer treated by radiation therapy: results of a multiple regression analysis. Radiother Oncol. 1995;35:107–17.
22. Perez CA, et al. Carcinoma of the uterine cervix: I. Impact of prolongation of overall treatment time and timing of brachytherapy on outcome of radiation therapy. Int J Radiat Oncol Biol Phys. 1995;32:1275–88.
23. Zarba JJ, et al. A phase I–II study of weekly cisplatin and gemcitabine with concurrent radiotherapy in locally advanced cervical cancer. Ann Oncol. 2003;14:1285–90.
24. Umanzor J, et al. Concurrent cisplatin/gemcitabine along with radiotherapy in locally advanced cervical carcinoma: a phase II trial. Gynecol Oncol. 2006;100:70–5.
25. Rose PG, et al. A phase I study of gemcitabine followed by cisplatin concurrent with whole pelvic radiation therapy in locally advanced cervical cancer: a Gynecologic Oncology Group study. Gynecol Oncol. 2007;107:274–9.
26. Puget Sound Oncology Consortium. Weekly gemcitabine and cisplatin in combination with pelvic radiation in the primary therapy of cervical cancer: a phase I trial of the Puget Sound Oncology Consortium. Gynecol Oncol. 2006;101:429–35.
27. Kim YS, et al. Prospective randomized comparison of monthly fluorouracil and cisplatin versus weekly cisplatin concurrent with pelvic radiotherapy and high-dose rate brachytherapy for locally advanced cervical cancer. Gynecol Oncol. 2008;108:195.
28. Pfaendler KS, et al. Changing paradigms in the systemic treatment of advanced cervical cancer. Am J Obstet Gynecol. 2016;214(1):22–30.
29. Barter JF, et al. Diagnosis and treatment of pulmonary metastases from cervical carcinoma. Gynecol Oncol. 1990;38(3):347–51.
30. Zola P, et al. Could follow-up different modalities play a role in asymptomatic cervical cancer relapses diagnosis? An Italian multicenter retrospective analysis. Gynecol Oncol. 2007;107(Suppl. 1 (1)):S150–4.
31. Carlson V, et al. Distant metastases in squamous cell carcinoma of the uterine cervix. Radiology. 1967;88(5):961–6.
32. Friedlander M, Grogan M, U.S. Preventative Services Task Force. Guidelines for the treatment of recurrent and metastatic cervical cancer. Oncologist. 2002;7(4):342–7.
33. Thigpen T, et al. Cis-dichlorodiammine platinum (II) in the treatment of gynecologic malignancies: phase II trials by the Gynecologic Oncology Group. Cancer Treat Rep. 1979;63(9–10):1549–55.
34. Ishikawa H, et al. Platelet-sparing effect of paclitaxel in heavily pretreated ovarian cancer patients. Int J Clin Oncol. 2002;7(5):330–3.
35. Kitagawa R, et al. Paclitaxel plus carboplatin versus paclitaxel plus cisplatin in metastatic or recurrent cervical cancer: the open-label randomized phase III trial JCOG0505. J Clin Oncol. 2015;33(19):2129–35.
36. Lorusso D, et al. A systematic review comparing cisplatin and carboplatin plus paclitaxel-based chemotherapy for recurrent or metastatic cervical cancer. Gynecol Oncol. 2014;133(1):117–23.
37. Moore DH, et al. Phase III study of cisplatin with or without paclitaxel in stage IVB, recurrent, or persistent squamous cell carcinoma of the cervix: a Gynaecologic Oncology Group study. J Clin Oncol. 2004;22(15):3113–9.
38. Monk BJ, et al. Phase III trial of four cisplatin-containing doublet combinations in stage IVB, recurrent, or persistent cervical carcinoma: a Gynecologic Oncology Group study. J Clin Oncol. 2009;27(28):4649–55.
39. McKim A, et al. An economic analysis of cisplatin alone versus cisplatin doublets in the treatment of women with advanced or recurrent cervical cancer. Eur J Gynaecol Oncol. 2016;37(3):353–6.
40. Moore DH, et al. Prognostic factors for response to cisplatin-based chemotherapy in advanced cervical carcinoma: a Gynecologic Oncology Group study. Gynecol Oncol. 2010;116(1):44–9.

41. No JH, et al. Expression of vascular endothelial growth factor and hypoxia inducible factor-1 alpha in cervical neoplasia. Ann N Y Acad Sci. 2009;1171:105–10.
42. Tang X, et al. Overexpression of human papillomavirus type 16 oncoproteins enhances hypoxia-inducible factor 1 alpha protein accumulation and vascular endothelial growth factor expression in human cervical carcinoma cells. Clin Cancer Res. 2007;13:2568–76.
43. Tewari KS, et al. Improved survival with bevacizumab in advanced cervical cancer. N Engl J Med. 2014;370(8):734–43.
44. Penson RT, et al. Patient reported outcomes in a practice changing randomized trial of bevacizumab in the treatment of advanced cervical cancer: an NRG Oncology/Gynecologic Oncology Group study. Lancet Oncol. 2015;16(3):301–11.
45. McLachlan J, et al. The impact of systemic therapy beyond first-line treatment for advanced cervical cancer. Clin Oncol. 2017;29:153–60.
46. Chan JK, et al. Prognostic factors in neuroendocrine small cell cervical carcinoma. Cancer. 2003;97:568–74. American Cancer Society
47. Lee JM, et al. Prognostic factors in FIGO stage IB-IIA small cell neuroendocrine carcinoma of the uterine cervix treated surgically: results of a multi-center retrospective Korean study. Ann Oncol. 2008;19:321.
48. Silva EG, et al. Small cell carcinoma of the uterine cervix: pathology and prognostic factors. Surg Pathol. 1989;2:105–15.
49. Chan JK, et al. Prognostic factors in neuroendocrine small cell cervical carcinoma: a multivariate analysis. Cancer. 2003;97:568.
50. Cohen JG, et al. Small cell carcinoma of the cervix: treatment and survival outcomes of 188 patients. Am J Obstet Gynecol. 2010;203:347.e1.
51. Chang TC, et al. Prognostic factors in surgically treated small cell cervical carcinoma followed by adjuvant chemotherapy. Cancer. 1998;83(4):712–8.
52. Zivanovic O, et al. Small cell neuroendocrine carcinoma of the cervix: analysis of outcome, recurrence pattern and the impact of platinum-based combination chemotherapy. Gynecol Oncol. 2009;112:590.
53. Eiriksson L, et al. Advancing fertility-sparing treatments in cervical cancer: where is the limit? Gynecol Oncol. 2012;126(3):317–8.
54. Lissoni AA, et al. A phase II, randomized trial of neo-adjuvant chemotherapy comparing a three-drug combination of paclitaxel, ifosfamide, and cisplatin (TIP) versus paclitaxel and cisplatin (TP) followed by radical surgery in patients with locally advanced squamous cell cervical carcinoma: the Snap-02 Italian collaborative study. Ann Oncol. 2009;20(4):660–5.
55. Li R, et al. Prognostic value of responsiveness of neoadjuvant chemotherapy before surgery for patients with stage IB2/IIA2 cervical cancer. Gynecol Oncol. 2013;128(3):524–9.
56. NACCCMA Collaboration, Tierney JF. Neoadjuvant Chemotherapy for Locally Advanced Cervical Cancer Meta-analysis Collaboration. Neoadjuvant chemotherapy for locally advanced cervical cancer: a systematic review and meta-analysis of individual patient data from 21 randomised trials. Eur J Cancer. 2003;39(17):2470–86.
57. Eddy GL, et al. Treatment of ("bulky") stage IB cervical cancer with or without neoadjuvant vincristine and cisplatin prior to radical hysterectomy and pelvic/para-aortic lymphadenectomy: a phase III trial of the Gynecologic Oncology group. Gynecol Oncol. 2007;106(2):362–9.
58. Mossa B, et al. Follow-up in a long term randomized trial with neoadjuvant chemotherapy for squamous cell cervical carcinoma. Eur J Gynaecol Oncol. 2010;31(5):497–503.
59. Dueñas-Gonzalez A, et al. A phase II study of multimodality treatment for locally advanced cervical cancer: neoadjuvant carboplatin and paclitaxel followed by radical hysterectomy and adjuvant cisplatin chemoradiation. Ann Oncol. 2003;14(8):1278–84.
60. Lissoni AA, et al. A phase II, randomized trial of neo-adjuvant chemotherapy comparing a three-drug combination of paclitaxel, ifosfamide, and cisplatin (TIP) versus paclitaxel and cisplatin (TP) followed by radical surgery in patients with locally advanced squamous cell cervical carcinoma: the Snap-02 Italian collaborative study. Ann Oncol. 2009;20(4):660–5.

61. Kim HS, et al. Efficacy of neoadjuvant chemotherapy in patients with FIGO stage IB1 to IIA cervical cancer: an international collaborative meta-analysis. Eur J Surg Oncol. 2013;39(2):115–24.
62. McCormack M, et al. A phase II study of weekly neoadjuvant chemotherapy followed by radical chemoradiation for locally advanced cervical cancer. Br J Cancer. 2013;108(12):2464–9.
63. clinicaltrials.gov NCT01566240 INTERLACE – induction chemotherapy plus chemoradiation as first line treatment for locally advanced cervical cancer. https://clinicaltrials.gov/ct2/show/NCT01566240?term=NCT01566240&rank=1
64. Dueñas-González A, et al. Phase III, open-label, randomized study comparing concurrent gemcitabine plus cisplatin and radiation followed by adjuvant gemcitabine and cisplatin versus concurrent cisplatin and radiation in patients with stage IIB to IVA carcinoma of the cervix. J Clin Oncol. 2011;29:1678.
65. Tangjitgamol S, et al. Adjuvant chemotherapy after concurrent chemoradiation for locally advanced cervical cancer. Cochrane Database Syst Rev. 2014;12:CD010401.
66. International Randomised Study (Australia and USA) OUTBACK trial sponsored by GOG and RCOG. http://www.rtog.org/LinkClick.aspx?fileticket=lU6JlzhBt7s%3d&tabid=290
67. Hanahan D, et al. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
68. Lawrence M, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature. 2013;499:214–8. PMID: 23770567
69. Melsheimer P, et al. DNA aneuploidy and integration of human papillomavirus type 16 E6/E7 oncogenes in intraepithelial neoplasia and invasive squamous cell carcinoma of the cervix uteri. Clin Cancer Res. 2004;10:3059–63.
70. Kanaan H, et al. Are virus-induced cancers more sensitive to checkpoint inhibitors? Future Oncol. 2016;12(23):2665–8.
71. clinicaltrials.gov NCT02488759 CheckMate358 – a study to investigate the safety and efficacy of Nivolumab in virus-associated tumors. https://clinicaltrials.gov/ct2/show/NCT02488759?term=NCT02488759&rank=1
72. Clinicaltrials.gov NCT02834013 Nivolumab and Ipilimumab in treating patients with rare tumors. https://clinicaltrials.gov/ct2/show/NCT02834013?term=NCT02834013&rank=1

Potential Biomarkers for Personalized Radiation Therapy for Patients with Uterine Cervical Cancer

13

Pablo Moreno-Acosta, Shyrly Carrillo, Oscar Gamboa, Diana Mayorga, Alfredo Romero-Rojas, Alexis Vallard, Chloe Rancoule, and Nicolas Magné

Despite screening campaigns, uterine cervical cancer (UCC) remains one of the most frequent and lethal neoplasms, especially in developing countries [1–3]. When diagnosed in advanced stages, locally advanced cancer, the concomitant chemotherapy associated with external beam radiation therapy (RT) and brachytherapy is considered the standard of care in the management of UCC. However, 30–40% of patients with similar prognostic factors do not respond in a similar way to

P. Moreno-Acosta (✉)
Research Group in Cancer Biology, National Cancer Institute, Bogotá, Colombia

Research Group in Radiobiology Clinical, Molecular and Cellular, National Cancer Institute, Bogotá, Colombia
e-mail: pmoreno@cancer.gov.co

S. Carrillo
Research Group in Cancer Biology, National Cancer Institute, Bogotá, Colombia
e-mail: scarrillo@cancer.gov.co

O. Gamboa
Unit of Analysis, National Cancer Institute, Bogotá, Colombia

Research Group in Radiobiology Clinical, Molecular and Cellular, National Cancer Institute, Bogotá, Colombia
e-mail: ogamboa@cancer.gov.co

D. Mayorga
Research Group in Radiobiology Clinical, Molecular and Cellular, National Cancer Institute, Bogotá, Colombia

A. Romero-Rojas
Group of Pathology Oncology, National Cancer Institute, Bogota, Colombia
e-mail: aromero@cancer.gov.co

A. Vallard · C. Rancoule · N. Magné
Department of Radiation Oncology, Institut de cancérologie de la Loire-Lucien Neuwirth, Saint-Priest en Jarez, France
e-mail: alexis.vallard@icloire.fr; cloe.rancoule@icloire.fr; nicolas.magne@icloire.fr

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_13

comparable standard treatments [3, 4]. In fact, it has been demonstrated that UCC cells that are characterized by an important capacity for repopulation during RT lead to the development of a tumor cell population resistant to radiation [3–5]. Some of the clinical investigations conducted in UCC point out that there is a significant association between the response to treatment and factors of the tumor phenotype, such as changes in gene expression, protein, and metabolism, which have also been considered in biological experiments aimed at evaluating the microenvironment of the tumor and molecular mechanisms that regulate it [6–12]. However, the underlying biological phenomenon and the reasons for its variability from one patient to another are still under study. Several causes of the variability in the efficacy of RT have been studied without convincing results, so the study and identification of prognostic and predictive biomarkers would be extremely useful in the selection of patients for appropriate personalized radiation oncology [3, 13].

Uterine Cervical Cancer

Uterine cervical cancer is one of the malignant neoplasms with the highest incidence and mortality in the world; they are calculated around 555,000/year, new ill, 85% of cases occur in Third World countries, and it is considered a marker of underdevelopment [14]. Statistically, cancers detected early have a survival of 90%, and only 10% do so in advanced or late stages.

The hypothetical model proposed for the invasive UCC tumor development includes characteristic phenotypes of the tumor microenvironment, such as hypoxia (low oxygen levels up to 1%), increased glycolysis, and acidosis (low pH) [13, 14]. Under these conditions, the malignant cells use alternative metabolic pathways to which they commonly use normal cells to meet their needs, adapt, and thus favor their proliferation and survival. UCC begins with the appearance of preneoplastic lesions in transformation of the epithelial tissue of the cervix and how a consequence of infection with HPV (human papillomavirus) [15, 16], which, maintained over time, continues to form carcinoma in situ, characterized by excessive cell proliferation, which gives rise to the hypoxic condition [17, 18]. This condition is conserved during the next stage of cellular invasion (invasive cancer), in which the glycolytic and acidosis phenotypes are evident. Together with these events, the expression of genes and proteins is modulated, some of them considered with possible prognostic value in UCC and in other types of cancer [19–22], such as those that we have studied in our work team: IGF -1R, IGF-I, IGF-II, GAPDH, HIF-1 alpha, survivin, GLUT1, CAIX, HKII, hTERT, and HPV16 variants [3, 18, 23–30].

Treatment

To consider the type of treatment that patients with preinvasive lesions and UCC should receive, the clinical classification of the tumor stage should be considered.

For preinvasive lesions (stage 0), conservative techniques that preserve a woman's fertility are used, such as laser surgery, conization (extraction of cone-shaped tissue), and electrosurgery. In these cases, the recurrence rate is low (10–15%), and progression to invasive disease is rare [31]. There are three types of treatment for invasive disease: surgery, exclusive radiotherapy, and radiotherapy concomitant to chemotherapy. Women in early stages IA1 and IA2 undergo hysterectomy (surgery to remove the uterus alone or with neighboring tissues depending on the case), abdominal or vaginal route. In advanced stages IB and IIA, the standard treatment is total radical hysterectomy given the affection to lymph nodes [31]. The survival rates with this type of treatment range between 80% and 90%. When the tumor exceeds 4 cm, exclusive radiotherapy is given. In advanced stages IIB, IIIA, IIIB, and IVA, the treatments with curative intention are the exclusive radiotherapy and the radiotherapy concomitant to chemotherapy and the survival rates oscillate between 40% and 70%. Patients who arrive at stage IVB receive exclusive radiotherapy with palliative intention [31, 32].

Oncology Radiation

Radiation therapy (RT) began to be used as of 1986, being an important component in the treatment of cancer, covering approximately 70% of all patients receiving radiation therapy during its course of the disease and contributing as a curative treatment for 40% (5). The high-energy radiation used in the RT removes tumor cells, by damaging the genetic material, blocking their ability to divide and proliferate [33].

Ionizing radiation is provided in fractions to reduce the risk of normal tissue injury and increase the therapeutic action in tumor tissue. These fractions are given in two sessions, teletherapy and brachytherapy [34]. The radiation is administered for healing; it is also used as a palliative treatment in cancer. Radiation therapy includes combination treatment strategies such as surgery, chemotherapy, immunotherapy [35], and targeted therapy, among others. Radiation as neoadjuvant therapy aims to reduce the tumor mass if used after surgery and as adjuvant therapy used after surgery and destroys the microscopic tumor cells [35]. Radiotherapy achieves its therapeutic effect through the induction of different types of cell death, including apoptosis or programmed cell death, mitotic cell death or mitotic catastrophe, necrosis, senescence, and autophagy. Radiation therapy does not kill malignant tumor cells immediately; it takes hours, days, or weeks of treatment before the malignant tumor cells begin to die, after which the cancer cells continue to die for weeks or months after the radiation therapy ends [35].

Radiotherapy modalities include fractionated radiotherapy, which is based on the radiobiological properties of cancerous tissues and normal tissues, with a typical radiation regimen now consisting of daily fractions of 1.5 to 3G and given for several weeks [35]. Among the technological advances, we find that 3D conformal radiotherapy (3DCRT), intensity modulated radiotherapy (IMRT), image-guided radiotherapy (IGRT), body stereotactic radiotherapy (SBRT) and photon radiation (X-ray and gamma rays) are widely used, as well as the radiation of particles

(electron beams, protons and neutrons) [35]. Along with the state-of-the-art radiotherapy and the use of established technologies, treatment has evolved with the use of radioactive sources located near or within tumors, electron radiation, and heavy ion radiation, such as protons and ions of carbon [36].

Resistance to Radiation Therapy

The poor responses to treatments and to radiotherapy leads to the development of innovative and effective therapies such as cancer of advanced, metastatic, and refractory cervix, an aspect that is a high priority, so molecular research and orientation is done and needed to identify new targets for therapy [3]. Resistance to the response of radiotherapy depends on different molecular factors, such as tissue oxygenation, activation of oncogenes, and loss of tumor suppressor genes and activated aberrant molecular signaling [3, 37]. The non-response to treatment could be explained by two reasons: the first is because cervical carcinomas are characterized by having a marked capacity to repopulate with dividing cells of rapid substitution of those that die by radiation or chemical agents [34]. The other possible explanation is related to the participation of different pre-existing factors that could be involved in the response to treatment, such as low levels of hemoglobin (hypoxic anemia), poor immune function, tumor status, low degree of differentiation, the microenvironment tumor that meets tumor hypoxia, increased glycolysis, and extracellular acidosis [34].

Recently, molecular agents focused on critical pathways of malignant transformation of the cervix have been evaluated in early clinical trials in combination with external beam radiation, heralding the era of concurrent bio-radiotherapy for locally advanced cervical cancer [38, 39]. The main strategy is to find and exploit the genetic or microenvironmental differences between normal and malignant tissues at the level of each patient, thus leading to the study and identification of prognostic and predictive biomarkers that would be extremely useful in the selection of patients. A prognostic biomarker provides prospective information and indicates the probable course of the disease in an untreated individual, to guide therapeutic decisions, and a predictive biomarker gives information on the probability of tumor response to a given therapy and allows the identification of subpopulations of patients who are more likely to respond [40]. The use of these biomarkers in the field of molecular orientation and individualization of radiation therapy could be focused on the modulation of DNA repair, cell cycle checkpoints, signal transduction pathways, tumor microenvironment (hypoxia, vascularization, glycolysis), and normal tissue damage [3]. Regarding signal transduction pathways or signaling cascades, knowing the main signaling pathways activated by IGF-IR because of binding with its ligands or external stimuli such as hypoxia, low levels of hemoglobin, ionizing radiation, or the presence of HPV16 variants, an approach model can be proposed which could explain resistance to radiation therapy (Fig. 13.1). Likewise, the activation of glycolytic pathways that provide the energy requirements would be associated with the activation of PI3K/Akt, activation to which hypoxia-inducible genes contribute, such as HIF-1 alpha, which in turn would stimulate the transcription of genes such as GAPDH, GLUT 1, IGF-II, and IGF1R [14]. The IGF1R would mediate resistance to

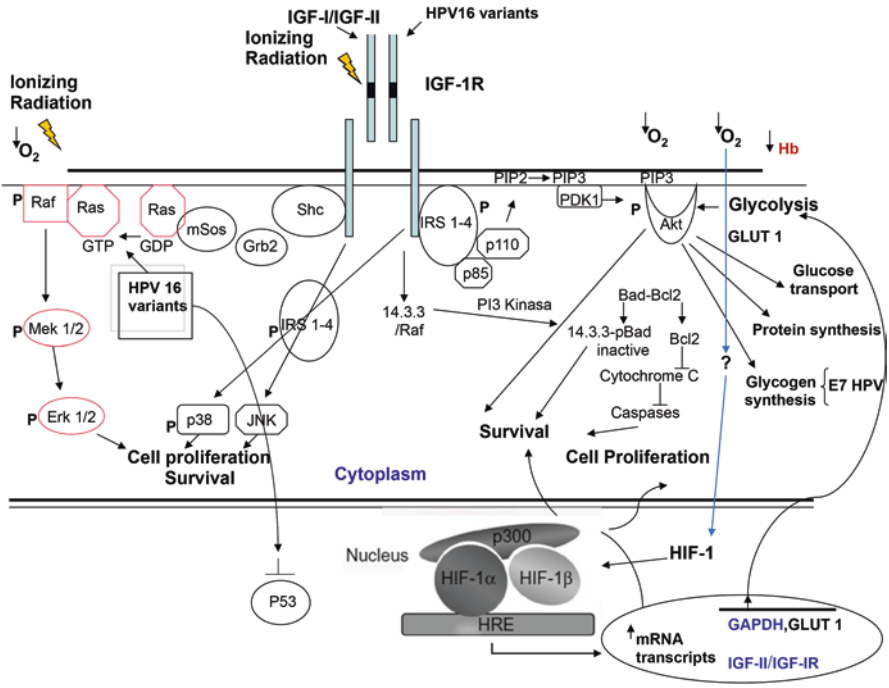

Fig. 13.1 Approach model to intracellular mechanisms that could explain resistance to radiation therapy

ionizing radiation through pathways such as PI3K/Akt and MAP kinases, pathways that can be shared in signaling effects by hypoxia. In this way, the transcriptional activation of IGF-II, IGF-IR, GAPDH, and GLUT 1 would regulate cell survival [14]. Regarding the role that HPV16 could play in cell survival, Vogt et al. reported that the survival of UCC cells (HPV (+)) depends on the inhibition of the p53/PUMA/Bax cascade, which is mediated by E6 [41]. The presence of the 350G variant (L83 V) of E6 of HPV16 according to that reported by Lichtig et al. could potentiate the degradation of p53, which would favor tumor cell survival [42]. Regarding non-European variants of HPV16, previous studies suggested that Asian-American variants induced the overexpression of IGF1R [3, 43, 44].

The exclusive RT that was sometimes performed in the present study provides a unique and "pure" model of radioresistance in UCC and could be the missing link between in vitro studies and state-of-the-art chemoradiotherapy studies that probably feature too many parameters to identify radioresistance causes [3, 27].

Biomarkers of Resistance to Radiation Therapy

Hypoxia and Hemoglobin Level The presence of hypoxia in solid tumors is a concern at the clinical level due to its negative impact on the prognosis and treatment response. HIF-1 alpha (hypoxia-inducible factor) is an endogenous marker associated with tumor hypoxia. The overexpression of HIF-1 alpha, a protein

induced by hypoxia that upregulates prosurvival and pro-proliferation signaling pathways, has been reported to be a predictive marker of response and prognosis in UCC treated with exclusive radiotherapy [21]. Moreno-Acosta et al. reported the overexpression of HIF-1 alpha in 74.1% from patients with UCC; in the association between the patient and the response to radiotherapy, 3 months after completion, the results of overexpression of HIF-1 alpha were not significant, nor was there any association with prognostic factors of progression-free survival and overall survival [3]. However in a multiple correspondence analysis, (Fig. 13.2), where two distinct groups could be identified, HIF-1 alpha overexpression was closely related to a group of patients who presented the following characteristics: incomplete response 3 months after treatment termination, cancer relapse, death, hemoglobin level (Hb) <11 g/dl, and treatment based on exclusive radiotherapy.

Previous experimental and clinical studies suggest that there is a direct association between the decrease in Hb levels and decreased oxygenation in a tumor [45]. In squamous cell carcinoma, like that of the cervix, the prognostic impact of anemia is well established [45]. Studies conducted by Moreno-Acosta et al. revealed that Hb levels <11 g/dl pretreatment were observed in the group of patients who did not

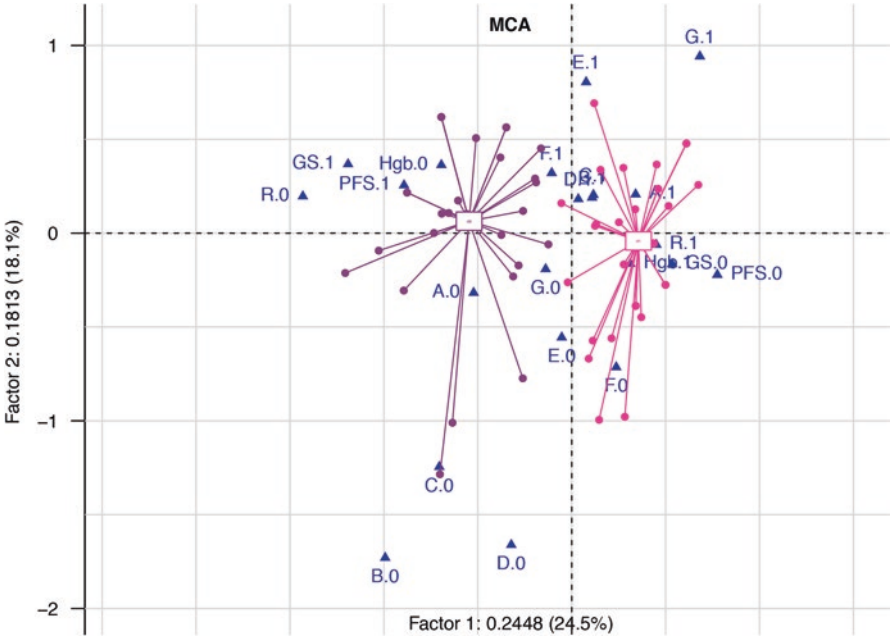

Fig. 13.2 Multi-correspondence analysis (n, 71 patients included, for which all information was available). Legend: A: type of treatment: radiochemotherapy (1) and radiotherapy (0); B: survivin; C: IGF1R-β; D: IGF1R-α; E: GLUT1; F: HIF1-α; G: CAIX. For these: expression was strong (1) or negative (0); hemoglobin (Hb): Hb > 11 g/dL (1); Hb ≤ 11d/dL (0); R0: non-responders; R1: responders; GS.0: alive GS1: death PFS.0: no relapse PFS.1: relapse. (From Moreno-Acosta et al. [3], with permission)

present complete response to exclusive radiotherapy; the results of the comparative analysis demonstrated a significant difference between the levels of Hb in patients with no response compared with the complete response group, and multivariate analysis revealed a close risk to the significance for patients with anemia that failed to respond to exclusive radiotherapy [7, 14–16]. This finding was consistent with reports that considered that anemia is a risk factor predictive of treatment outcome [7, 45], since it has been associated with an unfavorable local control of disease [7, 15, 45, 46] and low survival rates [45, 46]. Retrospective studies, similar to the present study, show that those patients with Hb levels <11 g/dl have a high risk of reducing DFS, which can be improved with the correction of the anemia [7, 15]. In studies reported by Moreno-Acosta et al., Hb (Hb ≤11 g/dl) was marginally correlated with reduced PFS and OS; interestingly, Hb was not significantly correlated with Karnofsky index, suggesting that the poor prognosis value of anemia could not only been seen through the prism of the performance status [3]. Furthermore, previous experimental and clinical studies suggested a direct association between anemia and a poor tumor oxygenation [20], limiting the radio-induced oxygen effect and therefore decreasing the efficacy of radiotherapy. In squamous cell carcinoma and especially in UCC, the prognostic impact of anemia is well established [3, 7, 20]. These findings suggest that, in addition to molecular biomarkers, hemoglobin levels could be a reliable, economical, and easily accessible biomarker to be considered in radiation resistance. Finally, although the association between hypoxia and poor response to RT has been widely described and known as a common cause of RT failure [46, 47], no efficient solution could be found yet to offer neoadjuvant treatment for patients with the highly hypoxic cancers, such as UCC.

IGF1R Gene Expression and IGF1R Protein Expression The insulin-like growth factor I receptor (IGF1R) is a ubiquitous growth receptor that may convey signals associated with radiation resistance. Through autocrine or paracrine stimulation with its ligands IGF1, IGF2, and insulin, IGF1R induces autophosphorylation and activation of specific tyrosine kinase residues, initiating signaling cascades such as Ras/Raf/mitogen-activated protein kinases (MAPK) and phosphoinositide 3-kinase (PI3K), which are downstream oncoproteins involved both in cell survival and resistance [4]. Moreover, previous studies have suggested that increased expression of IGF1R in mouse fibroblasts, in primary breast tumors, and in cell lines of prostate and cervical cancer may confer relative resistance to ionizing radiation. The mechanism underlying this radioresistance may implicate DNA repair and anti-apoptotic pathways [4]. Regarding the association between IGF1R gene expression and IGF1R protein expression and resistance to radiation therapy, several studies have been reported [3, 4, 12, 14]. The IGF1R gene expression was related to a 28.6 times higher risk of RT failure in UCC patients HPV16 (+), suggesting the IGF-1R expression to be a strong predictive marker of lack of response to radiotherapy [3, 4]. The frequency of protein expression of IGF1R alpha and beta was very similar; however, it was observed that only IGFIR beta significantly affected OS. However, such results on protein expression need confirmation in a larger cohort of patients [3]. These studies suggest an association between IGF1R expression and response

to radiotherapy and contribute to highlight the remarkable role of IGF1R in cervical cancer, which has already been described as a significant prognostic factor in cervical cancer [3].

Glycolysis

GLUT1 Crosstalk between glucose metabolism and hypoxia was suggested and could be the root of resistance to radiotherapy. Warburg demonstrated in 1927 that most of cancer cells predominantly produced energy by a high rate of anaerobic glycolysis [3]. Recently, it was suggested that cancer cells widely expressed glucose-carrying membrane proteins (GLUT-1, GLUT-7), increasing neoplastic cell metabolism [48, 49]. Thus, GLUT-1 was reported to be overexpressed in 47% of UCC cells [48]. Regarding data on efficacy, OS, and PFS prognostic factors, Moreno-Acosta et al. reported that the 5-year DFS and OS rates were 60 and 62.5%, respectively, among patients with GLUT1 expression [7]. By contrast, the DFS and OS were 75% and 60%, respectively, among those with no GLUT1 expression [7]. These authors also reported that GLUT1 overexpression was marginally correlated with reduced OS, with a median OS of 2.5 years for patients without overexpression vs. 1.9 years for the high-expression subgroup [3]. These findings suggested an effect of GLUT1 associated with response to treatment.

GAPDH GAPDH (glyceraldehyde 3 phosphate dehydrogenase) as a glycolytic enzyme in the cytoplasm, GAPDH also participates in many intracellular processes such as fusion at the membrane level, phosphotransferase activity, export of nuclear RNA, replication and repair of DNA, nitric oxide metabolism, and apoptosis and is also involved in neuronal disorders, in viral pathogenesis, and in some types of cancer [4]. An increase in the expression of GAPDH in hypoxic tissues, as the tissues of UCC, has also been reported to increase resistance to ionizing radiation [50]. In fact, high GAPDH levels may enhance glycolysis and meet the metabolic requirements of the tumor cells [51]. The UCC present higher levels of GAPDH expression compared to the control group (normal cervical tissue), and higher levels of expression of GAPDH were observed in patients co-expressing IGF2 and IGF1R, with hemoglobin levels $\leq$11 g/dl., highlighting the possible interaction between glucose metabolism and hypoxia-inducible factors [3]. This could indicate that IGF1R pathway activation may inhibit the transport of glucose across the plasma membranes through the downregulating effects of PI3K on GLUT1, GLUT3, and GLUT4 systems. Consequently, this could lead to an activation of the cellular glycolytic GADPH pathway [50]. GAPDH and IGF1R pathways may be both implicated in the tumoral cell glycolytic metabolism [4]. GAPDH and IGF1R routes can both be involved in the glycolytic metabolism of the tumor cell, which would contribute to survival and therefore to resistance to radiation.

HPV

Although human papillomavirus (HPV) has been demonstrated to be a causative factor of cervical cancer, its role as a modulating agent of response to treatment is

still unclear. Some studies suggested in very limited number of patients that HPV infection was associated with better prognosis of cervical cancer treated with radiation [44, 52]. In pilot studies, persistence of HPV after therapy was associated with poorer outcomes and increased local recurrences [44, 53, 54]. It was hoped that HPV genotyping could be a biomarker of response and prognosis in patients undergoing chemoradiotherapy, since HPV16 was reported as a possible predictor of poor response to radiotherapy [13]. However, results on the clinical impact of HPV and their prognostic significance remain controversial [13, 55–60]. Several HPV16 variants have been identified, as it is frequently involved in cervical carcinoma [61–63]. It was suggested that human papillomavirus variants might differently impact overall oncogenic process, affecting the virus assembly, the immune recognition, the p53 degradation, and finally the processes of cell immortalization [64, 65]. Thus, response to radiotherapy or to concurrent chemoradiotherapy might vary, depending on the involved HPV variants [65]. However, such hypotheses have never been confirmed, and HPV and especially HPV16 variants' role still need to be demonstrated in uterine cervical cancer. Interestingly, Kilic et al. suggested that HPV16 could interact with IGF-1R in cervical tumors, resulting in an increased radioresistance [3, 66]. Zacapala et al. reported that Asian-American variants of HPV16 induced the overexpression of IGF-1R [43, 44]. Therefore, it is likely that HPV16 variants do have an influence on response to radiotherapy. Human papillomavirus variants could be a molecular signature, reflecting different cellular particularities that can enhance radiation resistance. Functional studies based on the profile of each variant's altered genes are required to determine their significance in tumor biology. Research should also focus on recently evidenced HPV18 variants, since HPV18 is a major inducer of possibly radioresistant cervical cancers [67]. Elucidating the functional and pathological differences between variants of HPV18 and determining their relationship with radioresistance seem the topics of major interest [67]. Therefore, HPV16 variants could also be biomarkers of radioresistance, and antiviral drugs might act as agents restoring radiosensitivity [44, 68].

Customized Radiation Therapy Based on Molecular Targeting

Identifying biomarkers of radioresistance is therefore of primary interest since the standard treatment may be modified according to tumors' radioresistance status, testing radiosensitizing treatments only in patients with radioresistant tumors [3, 66]. However, targeted therapy development is a long and expensive process that often makes new anticancer drugs not affordable for transition countries. Original alternatives could be found, testing drugs already widely used for other non-cancer indications but clearly interfering with cancer-promoting elements, with interesting results particularly in glioblastoma [3, 69]. To our knowledge, such a process has never been performed in UCC. Curcumin (diferuloylmethane) is derived from the rhizome of the tropical plant *Curcuma longa*. It interferes with many cell processes, regulating the expression of inflammatory cytokines, growth factors, growth factor receptors, enzymes, adhesion molecules, apoptosis-related proteins, and cell cycle

proteins [69–71]. Curcumin has been recently described in preclinical studies as a natural inhibitor, "natural radiosensitizer" of IGF1Rβ and GLUT1 [69, 72, 73], and could be safely associated with chemotherapy and radiotherapy [74, 75]. A prospective phase II study will be designed in the near future, to evaluate the effect of curcumin as an inhibitor of IGF1Rβ and GLUT1 when given before radiotherapy.

Cidofovir is an antiviral drug that is effective against HPV [44, 76]. Most human papillomaviruses express E6 and E7 oncoproteins that can bind to p53 and retinoblastoma (pRb) tumor suppressor proteins and neutralize their function. Restoration of these pathways blocking E6 and E7 expression might provide a selective anticancer effect [44, 76]. In preclinical studies, cidofovir was shown reducing E6 and E7 expression in HPV-positive cervical carcinoma cells lines, and inducing an accumulation of active p53 and pRb, associated with an induction of cyclin-dependent kinase inhibitor p21 (WAF1/CIP1) [44, 76, 77]. Antiproliferative activity of cidofovir in HPV-treated cells was suggested, with S-phase cell cycle accumulation and concomitant decrease of cyclin A expression [44, 76, 78]. Stopping the activity of HPV-related oncoproteins and restoring "prodeath" proteins such as p53 could lead to a major selective radiosensibilization of cervical cancer cells, especially when infected by variants degrading p53 (such as European variants E-G350 and E-R10G). Thus, the therapeutic index of radiotherapy might be improved with reduced costs in patients with sensible HPV variants, thanks to antiviral agents. The encouraging results of a phase 1 study, showing no major toxicity and interesting efficacy when combining cidofovir with standard radiochemotherapy in stage IB2-IVA cervical cancer patients, raised high hopes [44, 78].

A major challenge for developed countries is probably to find costly acceptable molecules, making it possible to treat transition countries patients with efficient therapy combinations. This is, in the immuno- and highly personalized therapies age, more than ever, a challenge for humankind.

Conclusions

Studies about the identification of prognostic and predictive biomarkers in the path toward an adequate therapeutic management in radiation oncology of patients with UCC have been developed. These indicate the importance of future utility, of growth factors such as IGF1R, of factors related to hypoxia, such as HIF-1 alpha and hemoglobin levels, of components of glycolysis such as GLUT1 and GAPDH, and of the presence of variants of HPV16 as potential biomarkers. For these already considered therapeutic targets have been working on their modulation through the search, design, and development of inhibitors or radiosensitizers both synthetic and natural in the field of nanotechnology and nanomedicine.

Clinical trials should continue to be designed and developed in the short term, to evaluate the effect of natural inhibitors or radiosensitizers such as curcumin on IGF-1R and GLUT1. as well as the use of cidofovir as an HPV inhibitor, when administered before the treatment, and thus contribute to an appropriate therapeutic management as a personalized neoadjuvant treatment in UCC.

Acknowledgment The topic of revision includes a large part of the work we have been doing for several years and to which they contribute Functional Unit of Gynecology Oncology, Oncology Pathology Group, Group Area Radiotherapy Oncology, Unit of Analysis, which are part of the National Institute of Cancerology, Bogotá, Colombia, and the Department of Radiation Oncology, Institute de Cancérologie de la Loire-Lucien Neuwirth, Saint-Priest in Jarez, France.

References

1. Piñeros M, Cendales R, Murillo R, Wiesner C, Tovar S. Pap test coverage and related factors in Colombia, 2005. Rev Salud Publica (Bogota). 2007;9(3):327–41.
2. Lewis MJ, Council R, Sammons-Posey D. Barriers to breast and cervical cancer screening among New Jersey African Americans and Latinas. N J Med. 2002;99(1–2):27–32.
3. Moreno-Acosta P, Vallard A, Carrillo S, Gamboa O, Romero-Rojas A, Molano M, Acosta J, Mayorga D, Rancoule C, Garcia MA, Cotes Mestre M, Magné N. Biomarkers of resistance to radiation therapy: a prospective study in cervical carcinoma. Radiat Oncol. 2017;12(1):120.
4. Moreno-Acosta P, Gamboa O, Sanchez de Gomez M, et al. IGF1R gene expression as a predictive marker of response to ionizing radiation for patients with locally advanced HPV16- positive cervical Cancer. Anticancer Res. 2012;32:4319–26.
5. Yang J, Yue JB, Liu J, Yu JM. Repopulation of tumor cells during fractionated radiotherapy and detection methods (review). Oncol Lett. 2014;7(6):1755–60.
6. Huang Z, Mayr NA, Yuh WT, et al. Predicting outcomes in cervical cancer: a kinetic model of tumor regression during radiation therapy. Cancer Res. 2010;70(2):463–70.
7. Moreno-Acosta P, Carrillo S, Gamboa O, Romero-Rojas A, Acosta J, Molano M, Balart-Serra J, Cotes M, Rancoule C, Magné N. Novel predictive biomarkers for cervical cancer prognosis. Mol Clin Oncol. 2016;5(6):792–6.
8. Niibe Y, Watanabe J, Tsunoda S, et al. Concomitant expression of HER2 and HIF-1alpha is a predictor of poor prognosis in uterine cervical carcinoma treated with concurrent hemoradiotherapy: prospective analysis (KGROG0501). Eur J Gynaecol Oncol. 2010;31(5):491–6.
9. Noordhuis MG, Eijsink JJ, Roossink F, et al. Prognostic cell biological markers in cervical cancer patients primarily treated with (chemo) radiation: a systematic review. Int J Radiat Oncol Biol Phys. 2011;79(2):325–34.
10. Magne N, Chargari C, Deutsch E, et al. Molecular profiling of uterine cervical carcinoma: an overview with a special focus on rationally designed target based anticancer agents. Cancer Metastasis Rev. 2008;27:737–50.
11. Hockel M, Schlenger K, Aral B, Mitze M, Schaffer U, Vaupel P. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervical. Cancer Res. 1996;56:4509–15.
12. Lloret M, Lara PC, Bordón E, et al. IGF-1R expression in localized cervical carcinoma patients treated by radiochemotherapy. Gynecol Oncol. 2007;106:8–11.
13. Ferdousi J, Nagai Y, Asato T, et al. Impact of human papillomavirus genotype on response to treatment and survival in patients receiving radiotherapy for squamous cell carcinoma of the cervix. Exp Ther Med. 2010;1(3):525–30.
14. Moreno-Acosta P. Expresión del receptor de IGF-I y detección de variantes del virus del papiloma humano en pacientes con carcinomas escamocelualres invasivos de cuello uterino y su posible relación con la respuesta a la radioterapia [tesis Doctoral]. Bogotá (Colombia): Universidad Nacional de Colombia; 2006. 175 p.
15. IARC. Monographs on the Evaluation of Carcinogenic Risks to Humans. Human Papilomaviruses. Human Papillomavirus (HPV) Infection. Genomic Structure and Properties of Gene Products. 64, 40–43. 1995.
16. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189:12.

17. Haugland HK, Vukovic V, Pintilie M, Fyles AW, Milosevic M, Hill RP, et al. Expression of hypoxia-inducible factor-1alpha in cervical carcinomas: correlation with tumor oxygenation. Int J Radiat Oncol Biol Phys. 2002;53:854.
18. Hutchison GJ, Valentine HR, Loncaster JA, Davidson SE, Hunter RD, Roberts SA, et al. Hypoxia-inducible factor 1alpha expression as an intrinsic marker of hypoxia: correlation with tumor oxygen, pimonidazole measurements, and outcome in locally advanced carcinoma of the cervix. Clin Cancer Res. 2004;10:8405.
19. Moreno-Acosta P, Carrillo S, Gamboa O, Acosta Y, Balart-Serra J, Magne N, Melo-Uribe M-A, Romero-Rojas A-E. Expression of the hypoxic and glycolytic markers, CAIX, GLUT-1 and HKII and their association with early treatment response in squamous cell carcinomas of the uterine cervix. Prog Obstet Ginecol. 2013;56(8):404–13.
20. Moreno-Acosta P, Romero-Rojas A, Carrillo S, Gamboa O, Acosta J, Balart-Serra J, Magne N. GLUT1 and hemoglobin levels: hypoxic markers of treatment response in patients with locally advanced cervical cancer. Mol Cancer Ther. 2013;12(11 Suppl):C39.
21. Dayan F, Roux D, Brahimi-Horn MC, Pouyssegur J, Mazure NM. The oxygen sensor factor-inhibiting hypoxia-inducible factor-1 controls expression of distinct genes through the bifunctional transcriptional character of hypoxia-inducible factor-1alpha. Cancer Res. 2006;66:3688.
22. Gatenby RA, Gillies RJ. A microenvironmental model of carcinogenesis. Nat Rev Cancer. 2008;8:56.
23. Yang L, Cao Z, Li F, Post DE, Van Meir EG, Zhong H, et al. Tumor specific gene expression using the Survivin promoter is further increased by hypoxia. Gene Ther. 2004;11(15):1215–23.
24. Bache M, Holzapfel D, Kappler M, Holzhausen HJ, Taubert H, Dunst J, Hänsgen G. Survivin protein expression and hypoxia in advanced cervical carcinoma of patients treated by radiotherapy. Gynecol Oncol. 2007;104:139–44.
25. Mamede M, Higashi T, Kitaichi M, Ishizu K, Ishimori T, Nakamoto Y, et al. [18F] FDG uptake and PCNA, Glut-1, and hexokinase-II expressions in cancers and inflammatory lesions of the lung. Neoplasia. 2005;7:369.
26. Mendez LE, Manci N, Cantuaria G, Gomez-Marin O, Penalver M, Braunschweiger P, et al. Expression of glucose transporter-1 in cervical cancer and its precursors. Gynecol Oncol. 2002;86:138.
27. Loncaster JA, Harris AL, Davidson SE, Logue JP, Hunter RD, Wycoff CC, et al. Carbonic anhydrase (CA IX) expression, a potential new intrinsic marker of hypoxia: correlations with tumor oxygen measurements and prognosis in locally advanced carcinoma of the cervix. Cancer Res. 2001;61:6394.
28. Brahimi-Horn C, Pouyssegur J. The role of the hypoxia-inducible factor in tumor metabolism growth and invasion. Bull Cancer. 2006;93:E73.
29. Miller J, et al. HPV16 E7 protein and hTERT proteins defective for telomere maintenance cooperate to immortalize human keratinocytes. PLoS Pathog. 2014;9:e1003284.
30. Wellenhofer A, Brustmann H. Expression of human telomerase reverse transcriptase in vulvar intraepithelial neoplasia and squamous cell carcinoma: an immunohistochemical study with survivin and p53. Arch Pathol Lab Med. 2012;136(11):1359–65.
31. Instituto Nacional de Cancerología, Bogotá D. C Colombia. Cáncer de Cuello Uterino. En: Guías de práctica clínica en enfermedades neoplásicas. 413–428. 2001.
32. Betancourt Diego Palacio and Carlos Vicente rada Escobar. Anuario Estadístico. “Por el control del Cáncer”. Ministerio de la Protección Social, Instituto Nacional de Cancerología E.S.E. 4. 2007.
33. Garibaldi C, Jereczek-Fossa BA, Marvaso G, Dicuonzo S, Rojas DP, Cattani F, Starzyńska A, Ciardo D, Surgo A, Leonardi MC, Ricotti R. Recent advances in radiation oncology. Ecancermedicalscience. 2017;11:785.
34. Carrillo SA. Expresión de CAIX, GLUT-1 y HK II y su posible asociación con cáncer escamocelular invasivo de cuello uterino [tesis de Maestria]. Bogotá (Colombia): Universidad Nacional de Colombia; 2010. 90 p.
35. Baskar R, Lee KA, Yeo R, Yeoh KW. Cancer and radiation therapy: current advances and future directions. Int J Med Sci. 2012;9(3):193–9.

36. Chatterjee DK, Wolfe T, Lee J, Brown AP, Singh PK, Bhattarai SR, Diagaradjane P, Krishnan S. Convergence of nanotechnology with radiation therapy-insights and implications for clinical translation. Transl Cancer Res. 2013;2(4):256–68.
37. Vici P, Mariani L, Pizzuti L, et al. Emerging biological treatments for uterine cervical carcinoma. J Cancer. 2014;5(2):86–97.
38. Begg AC, Stewart FA, Vens C. Strategies to improve radiotherapy with targeted drugs. Nat Rev Cancer. 2011;11(4):239–53.
39. Mountzios G, Soultati A, Pectasides D, Dimopoulos MA, Papadimitriou CA. Novel approaches for concurrent irradiation in locally advanced cervical cancer: platinum combinations, non-platinum-containing regimens, and molecular targeted agents. Obstet Gynecol Int. 2013;2013:536765.
40. Brünner N. What is the difference between "predictive and prognostic biomarkers"? Can you give some examples? Connect. 2009;13:18–9.
41. Vogt M, Butz K, Dymalla S, Semzow J, Hoppe-Seyler F. Inhibition of bax activity is crucial for the anti-apoptotic function of the human papillomavirus E6 oncoprotein. Oncogene. 2006;25(29):4009–15.
42. Lichtig H, Algrisi M, Botzer LE, Abadi T, Verbitzky Y, Jackman A, Tommasino M, Zehbe I, Sherman L. HPV16 E6 natural variants exhibit different activities in functional assays relevant to the carcinogenic potential of E6. Virology. 2006;350(1):216–27.
43. Zacapala-Gómez AE, Del Moral-Hernández O, Villegas-Sepúlveda N, et al. Changes in global gene expression profiles induced by HPV 16 E6 oncoprotein variants in cervical carcinoma C33-A cells. Virology. 2016;488:187–95.
44. Moreno-Acosta P, Vallard A, Molano M, Huertas A, Gamboa Ó, Cotes M, Romero-Rojas A, Rancoule C, Magné N. HPV-16 variants' impact on uterine cervical cancer response to radiotherapy: a descriptive pilot study. Cancer Radiother. 2017;21(2):104–8. https://doi.org/10.1016/j.canrad.2016.09.018. Epub 2017 Mar 18.
45. Dunst J, Kuhnt T, Strauss HG, Krause U, Pelz T, Koelbl H, et al. Anemia in cervical cancers: impact on survival, patterns of relapse, and association with hypoxia and angiogenesis. Int J Radiat Oncol Biol Phys. 2003;56:778.
46. Vaupel P, Thews O, Hoeckel M. Treatment resistance of solid tumors: role of hypoxia and anemia. Med Oncol. 2001;18:243.
47. Mayer A, Hockel M, Vaupel P. Endogenous hypoxia markers: case not proven. Adv Exp Med Biol. 2008;614:127–36.
48. Airley RE, Loncaster J, Raleigh JA, Harris AL, Davidson SE, Hunter RD, et al. GLUT-1 and CAIX as intrinsic markers of hypoxia in carcinoma of the cervix: relationship to pimonidazole binding. Int J Cancer. 2003;104:85.
49. Lee WY, Huang SC, Hsu KF, Tzeng CC, Shen WL. Roles for hypoxia-regulated genes during cervical carcinogenesis: somatic evolution during the hypoxia-glycolysis-acidosis sequence. Gynecol Oncol. 2008;108:377.
50. Kim JW, Kim SJ, Han SM, Paik SY, Hur SY, Kim YW, Lee JM, Namkoong SE. Increased glyceraldehyde-3-phosphate dehydrogenase gene expression in human cervical cancers. Gynecol Oncol. 1998;71:266–9.
51. Hansen CN, Ketabi Z, Rosenstierne MW, Palle C, Boesen HC, Norrild B. Expression of CPEB, GAPDH and U6snRNA in cervical and ovarian tissue during cancer development. APMIS. 2009;117:53–9.
52. Harima Y, Sawada S, Nagata K, Sougawa M, Ohnishi T. Human papilloma virus (HPV) DNA associated with prognosis of cervical cancer after radiotherapy. Int J Radiat Oncol Biol Phys. 2002;52:1345–51.
53. Badaracco G, Savarese A, Micheli A, Rizzo C, Paolini F, Carosi M, et al. Persistence of HPV after radiochemotherapy in locally advanced cervical cancer. Oncol Rep. 2010;23:1093–9.
54. Song YJ, Kim JY, Lee SK, Lim HS, Lim MC, Seo SS, et al. Persistent human papillomavirus DNA is associated with local recurrence after radiotherapy of uterine cervical cancer. Int J Cancer. 2011;129:896–902.

55. Bachtiary B, Obermair A, Dreier B, Birner P, Breitenecker G, Knocke TH, et al. Impact of multiple HPV infection on response to treatment and survival in patients receiving radical radiotherapy for cervical cancer. Int J Cancer. 2002;102:237–43.
56. Kristensen GB, Karlsen F, Jenkins A, et al. Human papilloma virus has no prognostic significance in cervical carcinoma. Eur J Cancer. 1996;32A:1349–53.
57. Van Bommel PF, van den Brule AJ, Helmerhorst TJ, Gallee MP, Gaarenstroom KN, Walboomers JM, et al. HPV DNA presence and HPV genotypes as prognostic factors in low-stage squamous cell cervical cancer. Gynecol Oncol. 1993;48:333–7.
58. Lai HC, Sun CA, Yu MH, Chen HJ, Liu HS, Chu TY. Favorable clinical outcome of cervical cancers infected with human papilloma virus type 58 and related types. Int J Cancer. 1999;84:553–7.
59. Huang LW, Chao SL, Hwang JL. Human papillomavirus-31-related types predict better survival in cervical carcinoma. Cancer. 2004;100:327–34.
60. Tong SY, Lee YS, Park JS, Namkoong SE. Human papillomavirus genotype as a prognostic factor in carcinoma of the uterine cervix. Int J Gynecol Cancer. 2007;17:1307–13.
61. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003;348:518–27.
62. Moreno-Acosta P, Molano M, Huertas A, Sánchez de Gómez M, Romero A, González M, et al. A non-radioactive PCR-SSCP analysis allows distinguish between HPV 16 European and Asian-American variants in squamous cell carcinomas of the uterine cervix in Colombia. Virus Genes. 2008;37:22–30.
63. Huertas-Salgado A, Martin-Gamez DC, Moreno P, Murillo R, Bravo MM, Villa L, et al. E6 molecular of human papillomavirus (HPV) type 16: an updated and unified criterion for clustering and nomenclature. Virology. 2011;410:201–15.
64. Burk RD, Harari A, Chen Z. Human papillomavirus genome variants. Virology. 2013;445:232–43. https://doi.org/10.1016/j.virol.2013.07.018.
65. Hang D, Gao L, Sun M, Liu Y, Ke Y. Functional effects of sequence variations in the E6 and E2 genes of human papilloma virus 16 European and Asian variants. J Med Virol. 2014;86:618–26.
66. Kilic S, Cracchiolo B, Gabel M, Haffty B, Omar MO. The relevance of molecular biomarkers in cervical cancer patients treated with radiotherapy. Ann Transl Med. 2015;3(18):261.
67. Kaneko H, Yu D, Miura M. Overexpression of IGF-I receptor in HeLa cells enhances in vivo radioresponse. Biochem Biophys Res Commun. 2007;363:937–41.
68. Moreno-Acosta P, Cotes M, Gamboa O, Magné N. Radiotherapy and complementary treatment for cervical cancer. Int J Gynecol Cancer. 2015;25:1398.
69. Kast RE, Boockvar JA, Brüning A, et al. A conceptually new treatment approach for relapsed glioblastoma: coordinated undermining of survival paths with nine repurposed drugs (CUSP9) by the International Initiative for Accelerated Improvement of Glioblastoma Care. Oncotarget. 2013;4(4):502–30.
70. Shishodia S. Molecular mechanisms of curcumin action: gene expression. Biofactors. 2013;39(1):37–55.
71. Xiao Z, Zhang A, Lin J, et al. Telomerase: a target for therapeutic effects of curcumin and a curcumin derivative in Aβ1-42 insult in vitro. PLoS One. 2014;9:e1d1251.
72. Abouzeid AH, Patel NR, Rachman IM, Senn S, Torchilin VP. Anti-cancer activity of anti-GLUT1 antibody-targeted polymeric micelles co-loaded with curcumin and doxorubicin. J Drug Target. 2013;21(10):994–1000.
73. Gunnink L, Louters L. The mechanism of curcumin inhibition on GluT1. Available at: https://www.calvin.edu/academic/science/summer/2015posters_papers/GunninkPoster.pdf. Accessed 02/01/2016.
74. Mehta HJ, Patel V, Sadikot RT. Curcumin and lung cancer – a review. Target Oncol. 2014;9(4):295–310.
75. Higgins GS, Krause M, McKenna WG, Baumann M. Personalized radiation oncology: epidermal growth factor receptor and other receptor tyrosine kinase inhibitors, Mol Rad Oncol. Berlin/Heidelberg: Springer; 2016. p. 107–22.

76. Abdulkarim B, Sabri S, Deutsch É, Chagraoui H, Maggiorella L, Thierry J, et al. Antiviral agent cidofovir restores p53 function and enhances the radiosensitivity in HPV-associated cancers. Oncogene. 2002;21:2334–46.
77. Deberne M, Levy A, Mondini M, Dessen P, Vivet S, Supiramaniam A, et al. The combination of the antiviral agent cidofovir and anti-EGFR antibody cetuximab exerts an anti-proliferative effect on HPV-positive cervical cancer cell lines' in vitro and in vivo xenografts. Anti-Cancer Drugs. 2013;24:599–608.
78. Deutsch É, Levy A, Mazeron R, Gazzah A, Angevin EA, Ribrag V, et al. Phase I trial evaluating the antiviral agent cidofovir in combination with chemoradiation in cervical cancer patients: a novel approach to treat HPV-related malignancies? Eur J Cancer. 2014;50:74.

Radiotherapy for Uterine Cervical Cancer

14

Edward Chandy and Gemma Eminowicz

Uterine cervical cancer is an important health burden worldwide despite primary and secondary prevention measures in developed countries. Radiotherapy is a critical aspect of treatment with important roles in locally advanced disease as well as metastatic disease. A combination of external beam radiotherapy (EBRT) and brachytherapy, with chemotherapy if fitness allows, can be used to curatively treat locally advanced disease (FIGO stage IB1 to IVA). In the metastatic setting, radiotherapy to the primary tumor or metastases can effectively palliate symptoms such as pain or bleeding. EBRT uses photons to deliver radiation dose from a linear accelerator that is external to the patient, whereas brachytherapy uses radioactive material (e.g., iridium 192 for high-dose rate (HDR) brachytherapy) to deliver radiation dose to short distances from inside the patient. EBRT can be delivered using two-dimensional radiotherapy (2D-RT), three-dimensional conformal radiotherapy (3D-CRT), and intensity-modulated radiotherapy (IMRT) depending upon resources and skills available. This chapter describes the EBRT techniques currently used in the curative and palliative setting including the practical application, doses, evidence, and toxicities. Brachytherapy will also be detailed including image-guided brachytherapy.

E. Chandy
Clinical Oncology Department, Charing Cross Hospital, London, UK

G. Eminowicz (✉)
Department of Clinical Oncology/Radiotherapy, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, UK
e-mail: gemmaeminowicz@nhs.net

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_14

Cervical Cancer

Epidemiology

Cervix cancer is the fourth most common cancer in women and the seventh most common cancer worldwide. An estimated 266,000 women died from cervix cancer in 2012: 7.5% of all cancer deaths in women. Mortality varies across the globe with 87% of deaths occurring in less developed regions [1]. Access to radiotherapy also varies sharply with gross national income, and only 29% of low-income countries have functioning radiotherapy services [2]. Nonetheless, where available, radiotherapy is the treatment of choice for locally advanced cervical cancer.

Histology

Squamous cell carcinoma is the most common histological subtype of cervix cancer, accounting for 70–80%. Adenocarcinoma is the next most common (10–15%) [3]. Rarer subtypes such as small cell cancer are also seen. Both squamous cell and adenocarcinoma are driven by human papillomavirus (HPV) infection [4], and this, as in other tumor sites, is associated with radiosensitivity [5].

Staging

Despite advances in diagnostic imaging, clinical Federation of Gynecology and Obstetrics (FIGO) staging remains the global standard. Disease confined to the pelvis (i.e., up to FIGO stage IVA) is treated with radical radiotherapy. Small, locally confined tumors (FIGO stage IA and IB1) should be treated with surgery alone if patient fitness allows.

Lymphatic Spread and Risk of Disease

FIGO staging does not describe lymph node status, but, as expected, risk of lymph node increases with increasing stage (Table 14.1). Computed tomography (CT),

Table 14.1 Risk of lymph node involvement according to FIGO stage

Stage	Risk of pelvic LN metastasis (%)	Risk of PA LN metastasis (%)
1A1	<1	<1
1A2	3–6	<1
IB2	15–20	5–10
IIB	30	15
IIIB	50	30
IVA	80	50

magnetic resonance imaging (MRI), and ^{18}-fluorodeoxyglucose positron emission tomography (^{18}FDG-PET) should therefore complement clinical staging. Lymph node involvement is an indication for radiotherapy rather than surgery. Distant lymphadenopathy, i.e., nodal disease above the renal vessels, precludes the use of radical radiotherapy. Standard modern imaging modalities rely primarily on size criteria to assess lymphadenopathy. Even FDG-PET, which identifies abnormal metabolically active nodes, has a sensitivity of only up to 84% compared to surgical excision biopsy [6].

Development of Radiotherapy in Cervix Cancer

In 1906 Amand Routh, a gynecologist working at Charing Cross Hospital, London, published the first case report of cervix cancer treated with radiation. Its benefits over surgery, namely, reduced periprocedural mortality and efficacy in locally advanced malignancies, were immediately recognized, and, within a decade, case series were published documenting cure in inoperable cases [7].

Modern radiotherapy treatment can be delivered using external beam radiotherapy (EBRT) and internal brachytherapy (BT). Dose is measured in gray (Gy) which is calculated as joules of energy/kilogram. Linear accelerators are used to deliver multiple treatments, i.e., a fractionated course, of EBRT to the cervix, pelvis, or sites of metastatic disease, and a radioactive source, for example, iridium 192 in high-dose rate (HDR), is used to deliver brachytherapy internally to the cervix and uterine canal. In the radical cervical cancer (CC) setting, these modalities are combined to achieve optimal dosimetric results.

Radiobiology

Ionizing radiation achieves cell kill by inducing irreparable DNA strand breaks via the generation of free radicals. The radiation prescription aims to deliver a high dose to tumor and areas at risk of microscopic disease while keeping doses to normal structures within known radiobiological tolerance. The probability of tumor control and normal tissue toxicity is determined principally by the biologically effective dose (BED) and the total time over which treatment is given. BED is determined by the total energy delivered, the fractionation size, and the α/β ratio of the treated tissues.

The α/β ratio is a key radiobiological concept. It describes the linear-quadratic model, which predicts and explains the effect of fractionation on cell kill and can be determined empirically. α represents the linear portion of a cell survival curve and models the effect of a *single* electron causing a lethal double-strand DNA break. β forms the quadratic component of the curve. It stands for sensitivity to high-dose radiation, whereby *two* electrons induce two discrete single-strand breaks, adjacent in time and space to produce a lethal double-strand break.

The α/β ratio of the tumor is around 10 Gy, while the α/β ratio of late tissue reactions is lower at 2–3 Gy. Delivery of multiple small doses (fractionation) exploits this therapeutic window. BED can be expressed as EQD_2, which is the equivalent dose stated in 2 Gy fractions.

The Linear-Quadratic Model of Radiobiology

- $EQD_2 = D(d + \alpha/\beta)/(2+ \alpha/\beta)$
- $BED = D \times 1 + (d/ \alpha/\beta)$

EQD_2 = BED in 2 Gy per fraction equivalent; D = Total Dose; d = Dose per Fraction; BED = Biologically effective Dose

Since 1975 [8] the concept of the 4 "R"s of radiobiology has been widely accepted to explain the outcomes of fractionated treatment. The differential between normal and cancerous cells in their rates of *r*epair, *r*eassortment into radiosensitive phases of the cell cycle, *r*epopulation between fractions, and *r*eoxygenation during treatment allows oncologists to kill malignant tissue while preserving the function of adjacent normal structures. (Rod Withers' original paper described 4 Rs, and a 5th, radiosensitivity, was later adopted by the radiotherapy community after research in the early 1980s showed that tumors had different cell survival slopes after irradiation.) These principles underpin some of the treatment strategies discussed in this chapter.

Radiotherapy Techniques

Dimensional Radiotherapy (2D-RT)

2D-RT uses X-rays to conventionally simulate treatment if cross-sectional imaging is not available. The patient is clinically examined in the treatment position. The lower border of vaginal disease or the level of introitus is marked with a radiopaque marker. Anterior-posterior and lateral X-ray images are then taken. The field borders are defined on these X-rays according to anatomy as per Table 14.2 and Fig. 14.1. Diagnostic imaging can aid adaptation of borders to ensure adequate target coverage. Shielding can be added over the posterior sacrum on lateral X-ray and

Table 14.2 Anatomical borders of pelvis only RT field edges when using 2D-RT

Border	Anatomical position (four-field)
Superior	L4/5
Inferior	3 cm below vaginal disease (inferior obturator foramen)
Lateral	1–2 cm lateral to pelvic brim
Anterior	1 cm anterior to pubic symphysis
Posterior	S2/3 (entire sacrum if uterosacral ligament involved)

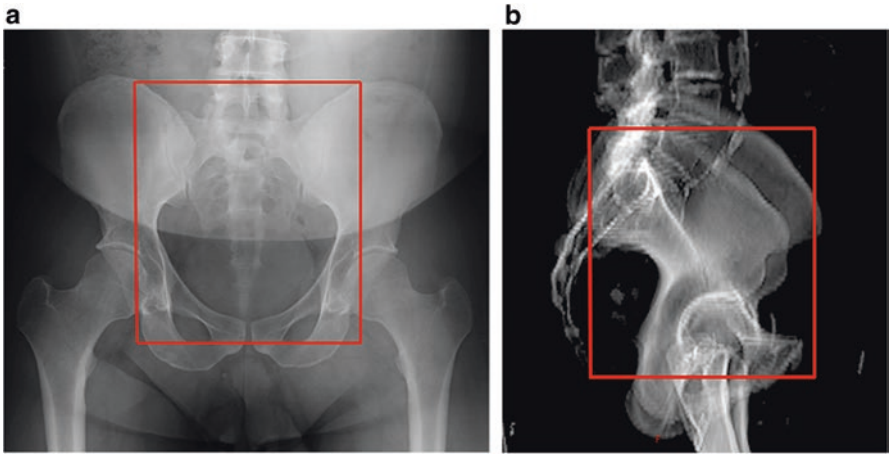

Fig. 14.1 Cervical pelvic RT treatment fields on anterior-posterior and lateral X-rays

small bowel superior laterally on the anterior-posterior X-ray. These fields are then used to create a four-field brick arrangement.

Three-Dimensional Conformal Radiotherapy (3D-CRT)

If available, 3D-CRT is preferred as 2D-RT has a high risk of unnecessary normal tissue irradiation. Patients undergo planning and treatment immobilized by knee and ankle supports with their arms on their chests or above their head. Skin tattoos are used to aid setup reproduction. A planning CT is acquired, traditionally with the bladder filled to achieve a comfortable, reproducibly full bladder. Some centers scan with a full and empty bladder to determine internal movement of the cervix and may deliver treatment according to bladder size, i.e., image-guided adaptive radiotherapy. Intravenous (IV) contrast is used to facilitate visualization of blood vessels.

A typical EBRT dose to the whole pelvis is 40–50.4 Gy in 20–28 fractions. Involved pelvic and para-aortic lymph nodes should ideally receive up to 60 Gy in 28 fractions using a simultaneous integrated boost (SIB), discussed later [9].

The planning scan is used, with additional information from the clinical examination and diagnostic imaging, to contour the clinical target volume (CTV) and the organs at risk (OARs). The principal OARs are the rectum, small bowel, bladder, and femoral heads. The CTV includes the tumor, the entire cervix and uterus, ovaries, fallopian tubes, bilateral parametrium, upper vagina, as well as involved lymph nodes and at-risk lymph node regions. Nodal regions are localized by their proximity to major blood vessels and known relapse patterns. Based on studies using ultrasmall particles of iron oxide as an MRI contrast agent, a margin of 7 mm around blood vessels (with editing for natural barriers to local spread, e.g., muscle and bone) allows coverage of almost 90% of nodes with micrometastatic involvement and minimizes dose to normal structures [10]. Common iliac, internal and external iliac, and upper presacral and obturator nodes should be included. The para-aortic

(PA) nodal strip is included if common iliac and/or PA nodes are positive for disease. Once the CTV is outlined, a margin is applied to account for internal organ motion and daily setup error resulting in a planning target volume (PTV). Usually a four-field beam arrangement is then applied with manual adjustments of beam weight, geometry, and addition of wedges to produce a "four-field brick" (Fig. 14.2).

IMRT

In recent years intensity-modulated radiotherapy (IMRT) has superseded standard 3D conformal radiotherapy. IMRT uses nonuniform beam fluences and complex beam arrangements to produce highly conformal dose coverage. The prescription dose and dose constraints for PTV and OARs are defined, and the planning algorithm software, most commonly using inverse planning, calculates the optimum arrangements. This allows good PTV coverage while sparing normal tissue [11].

Mundt et al. showed that whole pelvic IMRT reduced acute and chronic toxicity compared to 3D-CRT plans. Their planning technique allowed a halving of small bowel volume treated to prescription dose, with rectal and bladder volumes decreased by almost a quarter. No patient in this cohort developed Grade 3 acute toxicity. Grade 2 GI and GU toxicity was reduced from 91% to 60% and from 20% to 10%, respectively, in comparison to patients treated with traditional conformal plans at their center [12, 13] (Fig. 14.3).

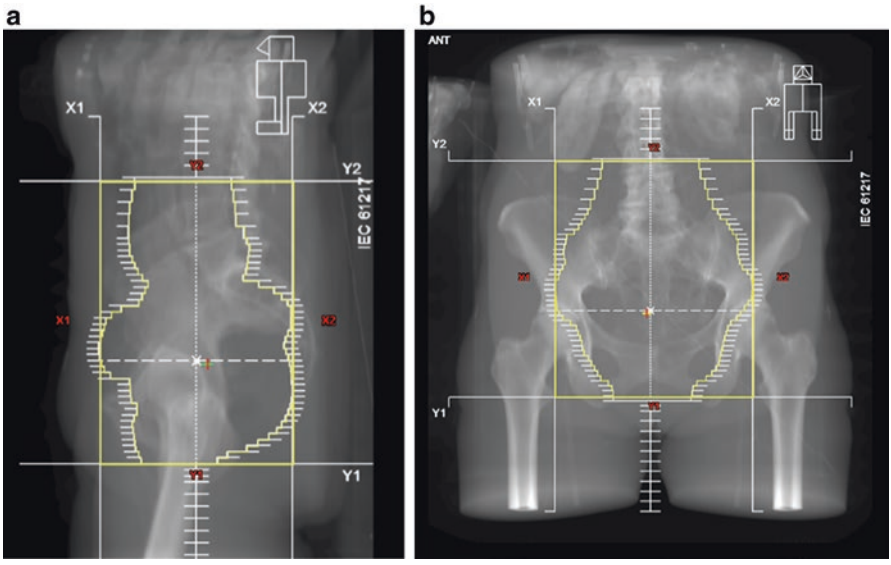

Fig. 14.2 Typical 3D conformal radiotherapy plan to pelvis planned as four-field brick with multi-leaf collimators (MLCs) to achieve shielding to normal structures

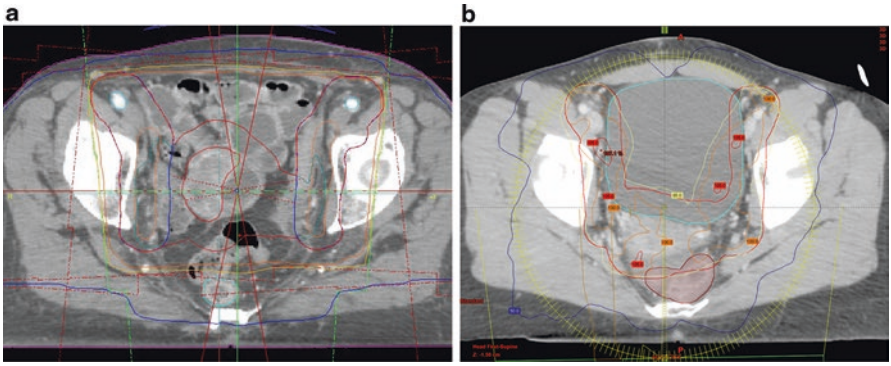

Fig. 14.3 Transverse CT images showing the dose distribution of 3D-CRT (a) versus IMRT (b) for cervical RT. Yellow line = 95% prescribed dose, orange = 100%, red = 105%, blue = 50%. Red structure arrowed (u shaped) = PTV. The IMRT plan (b) conforms better with concavity anteriorly compared to the 3D-CRT (a) box shape

Concurrent Chemoradiotherapy

Chemotherapy, most commonly weekly cisplatin, is used during radiation as a radiosensitizer and confers an extra 6% survival benefit according to a Cochrane meta-analysis [14]. A typical chemotherapy prescription is five weekly cycles of 40 mg/m^2 (max 70 mg) of concurrent cisplatin.

Brachytherapy

Brachytherapy (BT) is the use of a radioactive source delivering short-distance radiation in or directly next to the tissues being therapeutically irradiated. It permits high radiation doses to be used with precise anatomical distribution and is used as an adjunct to pelvic EBRT. For many years BT has been used with EBRT to treat the primary tumor as a highly conformal "phase II boost."

Bowel preparation must be considered and can be in the form of an enema prior to applicator insertion or insertion of flatus tube for the duration of planning and treatment. The patient receives a general anesthetic before a pelvic examination is undertaken to reassess the tumor. A urinary catheter is inserted prior to the placement of an intrauterine applicator with ring or ovoids at the vaginal fornices as seen in Fig. 14.4. Vaginal packing with gauze can secure the position of the applicator in close contact with the cervix and tumor. Imaging with CT or X-rays confirms the applicator position. A radioactive source is then inserted through the applicator channels, usually robotically driven with remote afterloading, stopping at predetermined dwell positions at specified times to deliver the prescribed dose. A predetermined pear-shaped dose distribution is delivered to a specified point. This point is "Point A" defined as 2 cm along the axis of the intrauterine canal and 2 cm lateral. The rapid dose fall-off allows adjacent normal tissue structures to be spared (Fig. 14.5).

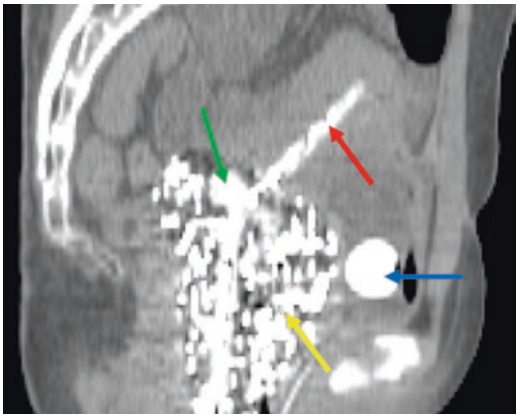

Fig. 14.4 Sagittal CT of intrauterine tube (red arrow) and ring (green arrow) positioned for BT with packing in vagina (yellow arrow) and urinary catheter balloon (blue arrow)

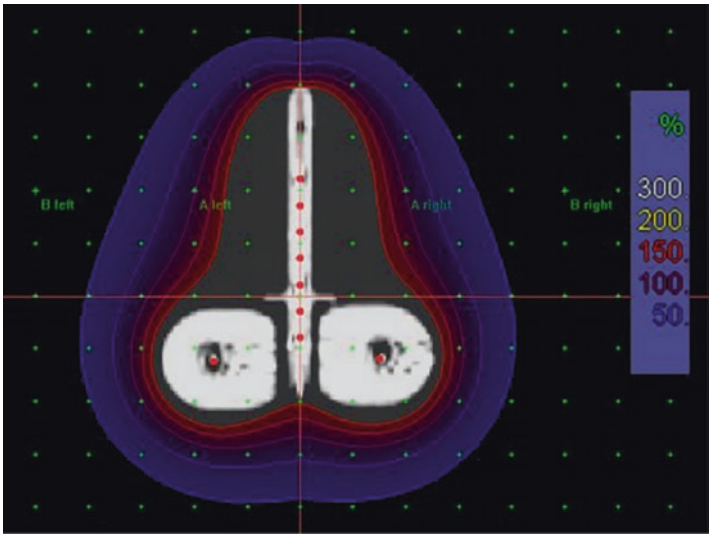

Fig. 14.5 Standard BT "pear-shaped" dose distribution prescribed to Point A. Green dots represent 1 cm distance (intrauterine tube is 4 cm long); red dots are dwell positions of radiation source. Points A and B are labeled, left and right. Colored isodoses represent percentage of prescribed dose ranging from 50% to 300%

Total (BT + EBRT) EQD2 doses of at least 80–85 Gy should be achieved. BT is administered after or toward the end of EBRT to allow tumor shrinkage and therefore smaller BT treatment volumes. BT can be delivered with varying dose rates. Low-dose-rate (LDR) BT is defined as doses 0.4–2 Gy/hr. High-dose-rate (HDR) BT is defined as dose >12 Gy/hr. Medium-dose rate (MDR) is the term used for doses between these ranges but is rarely used. In terms of outcome, they appear to be nearly equivalent, but HDR is becoming standard across much of the world as it has significant practical advantages primarily due to shorter treatment times [15, 16].

MR-Guided Brachytherapy

MRI-guided BT (or image-guided adaptive BT (IGABT)) has become the gold standard of care in recent years. The GYN GEC-ESTRO working group have standardized IGABT by providing evidence-based recommendations and universally recognized definitions. It remains logistically and technically demanding and is only feasible in specialist centers.

An MRI-compatible applicator is used with the option to insert interstitial needles into the parametria, with or without ultrasound guidance. Optimal placement of the applicator is crucial. Poor placement results in reduced disease-free survival [17]. An MRI scan is then performed to allow delineation of target structures (Fig. 14.6).

The GYN GEC-ESTRO guidance describes the high-risk CTV (HRCTV) to include macroscopic disease and the intermediate-risk CTV (IRCTV) and low-risk CTV (LRCTV) which designates areas at high or potential risk of microscopic disease, respectively. HRCTV is defined as the gross tumor visible on T2-weighted MRI or palpable on clinical examination at the time of BT, the whole cervix and any residual pathologic tissues after EBRT. IRCTV is defined as the HRCTV plus an anisotropic margin of up to 5–15 mm to cover the anatomical extent of the disease prior to chemoradiation. The LRCTV is not targeted during BT treatment.

A Point A plan is then applied to the imaging, and the dwell positions and timings are adapted to optimize HRCTV coverage and reduce OAR dose.

Dose coverage can be defined as the minimum dose delivered to a volume of interest. For IGABT the prescription is to D90 of HRCTV, which is the minimum dose delivered to 90% of this volume. Alternatively, a volume receiving a certain dose (in absolute terms or as a percentage) can be stated: the V100 or V60 Gy are the volumes receiving 100% of the prescribed dose or 60 Gy, respectively [18].

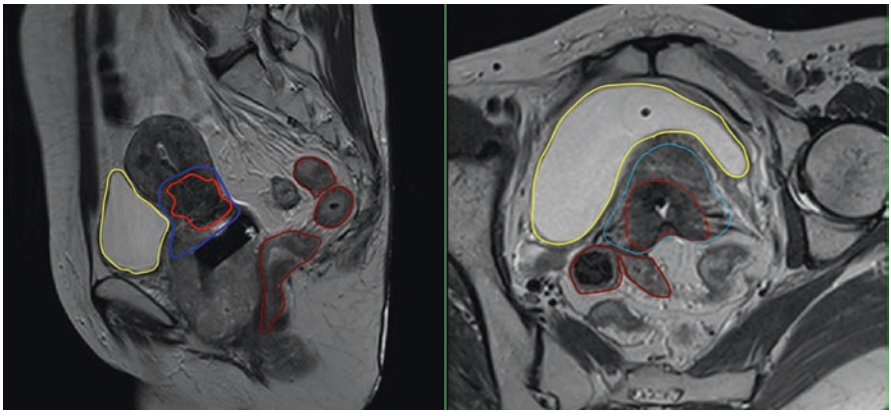

Fig. 14.6 MR-guided BT plan with CTV and OARs (rectum and bladder) delineated

Management of Cervix Cancer with Radiotherapy

Localized Disease

Standard of care for stage IA disease is surgery. Radical radiotherapy offers equal survival outcomes to surgery in stage IB–IIA disease though with less morbidity. A paper published in the Lancet in 1997 [19] randomized 343 patients aged 30–70 years to either surgery or radiotherapy (EBRT and BT without concomitant chemotherapy) and showed that 5-year overall and disease-free survival rates were statistically equivalent at 83% and 74%, respectively. A post hoc analysis suggested that adenocarcinoma did better with surgery than radiotherapy. It must be noted that 64% of the women assigned to surgery required adjuvant postoperative radiotherapy, and this combination of treatments had a higher rate of associated morbidity.

Tumor size, lymphovascular space invasion (LVSI), and deep stromal invasion have been shown to predict local recurrence. The GOG 92 2006 phase III trial randomized patients with stage IB disease with these poor prognostic features to postoperative pelvic RT or observation alone. The adjuvant RT arm showed a 46% reduction of recurrence. Postoperative RT was most beneficial in patients with adenocarcinoma or adenosquamous histology [20]. A 2012 Cochrane review [21] pooled this trial with a German randomized controlled trial [22] and concluded that although progression-free survival was improved, overall survival was not affected by adjuvant RT in stage 1B disease.

There is evidence suggesting that concurrent chemotherapy improves survival when given concurrently with postoperative RT but with higher rates of Grade 4 hematological and gastrointestinal toxicity [23].

Given the greater morbidity of using combined surgery and RT [19], accurate pre-treatment staging is vital. Where surgery alone is unlikely to be sufficient, for example, in localized tumors greater than 4 cm with high-grade differentiation and lymphovascular space invasion, primary chemoradiation should be used.

Locally Advanced Disease

Patients with locally advanced disease standardly receive combined chemoradiation and BT as surgery combined with radiotherapy has unacceptable toxicty [24].

A SEER database analysis published in 2013 showed that between 1998 and 2009, utilization of BT fell from 83% to 58%. The same analysis showed that BT was independently associated with a 12% improvement in overall survival [25]. BT is therefore a vital component of curative primary radiation for locally advanced cervical cancer.

RetroEMBRACE [26] was a multicenter retrospective cohort study examining outcomes in centers performing IGABT in locally advanced disease. Seven hundred and thirty one patients were followed up for a median of 43 months. All patients had histologically confirmed cervix cancer and were receiving definitive EBRT +/− concurrent chemotherapy followed by IGABT. The primary end point was local

control with overall survival as a secondary end point. Mean EBRT dose was 46 Gy and 77.4% received concurrent chemotherapy. Eighty-one percent of patients had MR-guided BT with the rest receiving CT-guided BT alone. Twenty-three percent of patients had combined interstitial and intracavitary BT. The mean D90 dose to HRCTV and IRCTV was 87 and 69 Gy, respectively. See Table 14.3 for 5-year local control.

RetroEMBRACE showed that tumor control probability was dose dependent [26]. Escalating dose from 75 Gy to 85 Gy resulted in a 3–7% increase in local control (depending on HRCTV volume). Further dose escalation to 90–95 Gy is predicted to increase local control by 1–4%. There is data to suggest that doses of 90 Gy can be achieved in up to half of patients within constraints of normal tissue tolerance. The data also suggested that in patients with smaller tumors, there is scope for de-escalation of dose, and this is to be tested in EMBRACE2. Interestingly RetroEMBRACE suggests that concomitant chemotherapy may be less important when using modern IGABT techniques [26].

Para-aortic Disease

Lymph node involvement confers a poorer prognosis and strongly correlates with 5-year overall survival [27]. The risk of para-aortic lymphadenopathy increases with more advanced disease, ranging from 5% to 10% in IB2 disease up to 50% in IVa disease [28] (see Table 14.1). Para-aortic disease up to the level of the renal vessels can be encompassed in a radical radiotherapy field using IMRT with acceptable late toxicity [29].

Integrated IMRT Boost for Involved Lymph Nodes

The DEPICT phase I/II trial completed recruitment in 2016. It recruited 44 patients to a multicenter dose-escalation study of SIB to involved lymph nodes [30]. Previous work has had encouraging results and allowed macroscopic nodal disease to receive higher doses of conformal radiotherapy without extending overall treatment time or unacceptable OAR toxicity [31]. The DEPICT results are yet to be published.

Treatment Time

As in other tumor sites, total treatment should be minimized in order to obtain optimum radiobiological efficacy. Repopulation by clonogenic tumor cells necessitates higher doses of radiation to achieve local control. There is evidence that cell kill by

Table 14.3 RetroEMBRACE outcomes according to FIGO stage

FIGO stage	5-year local control (%)	5-year overall survival
IB	98	83
IIA	94	NR
IIB	91	70
IIIA	71	NR
IIIB	75	42
IVA	76	NR

radiation (or chemotherapy) can result in accelerated repopulation, and this phenomenon has been seen most consistently in squamous cell tumors including cervix carcinoma [32]. A retrospective study showed that 3-year local recurrence was 26% vs. 9% for stage IB2 to IIIB disease when total treatment time was greater or less than 56 days, respectively [33]. Other retrospective studies have found that local control rates were diminished by 0.3–1.6% for every day of treatment prolongation [34, 35]. RetroEMBRACE demonstrated that time is as crucial to local control as total dose with an extra week of treatment time being equivalent to approximately a 5 Gy dose reduction [26]. The Royal College of Radiologists advises overall treatment time to be less than 56 days [9].

Tumor Hypoxia

Steps to improve tumor oxygenation may improve outcomes with radiotherapy. Retrospective studies dating from the 1960s demonstrate that anemia is associated with a poorer outcome in cervix cancer [36]. Whether this is a causal association is unclear, but radiation oncologists have traditionally used blood transfusions to maintain the hemoglobin above 12 g/dl during radiotherapy. There is little good randomized evidence to support this, but retrospective data suggests correcting anemia may improve survival [37]. Furthermore, there is a theoretical concern that blood transfusions may stimulate endogenous growth factors that promote tumor growth [38].

Smoking is etiologically implicated in cervix cancer and may also reduce tumor oxygenation. It increases the likelihood of acute and chronic radiation toxicity, and patients should be encouraged to stop smoking before treatment [39, 40]. The use of hyperbaric oxygen, erythropoietin, and other techniques for improving oxygenation of tumors during radiotherapy has not shown significant clinical benefit.

Recurrent Disease

Up to one third of women will suffer progressive or recurrent disease after primary treatment. The relapse rate is 11–22% in FIGO IB–IIA disease and 28–64% in IIB to IVa disease [41]. Its management depends on the site of recurrence and previous treatment. Recurrence within the pelvis is the most common site of failure and can be either central or lateral. Extra-pelvic disease commonly involves the para-aortic lymph nodes, lungs, liver, and bone. The majority of patients who recur will do so in the first 2 years after treatment and generally have a poor prognosis [42].

Tumor stage, LVSI, involved lymph nodes, and positive margins are all risk factors for recurrence after radical hysterectomy. 10–20% of patients develop recurrence after surgery and 75% of these will have isolated pelvic disease. These patients can be managed with chemoradiation. The morbidity of chemoradiation is higher in patients who have had previous surgery, and lower doses of radiation are therefore often used, e.g., 45 Gy in 25 fractions [42].

Patients treated surgically can be salvaged with RT at the time of recurrence if the disease is localized. One hundred and thirty patients treated at the Christie Hospital achieved a 40.2% 5-year overall survival, ranging from 55.4% of women

with vault recurrence down to 12.5% in women with lymph node relapse. All patients received four-field conformal RT +/− vaginal BT without chemotherapy. Grade 3 toxicity was only 1.1% [43]. Current practice is to treat with concurrent cisplatin and therefore outcomes are likely to be better [9]. IMRT facilitates sparing of normal tissue, and SIB and/or vault brachytherapy can be used to boost macroscopically involved areas of disease.

Patients treated with primary pelvic RT can subsequently receive PA nodal RT if the PA nodes are the only site of recurrence. Chou et al. [44] published a case series of 19 patients treated with RT or cisplatin-based RT to the PA strip to a dose of 45 Gy in 25 fractions. Five-year survival was 51.2% in those who received chemoradiation and 0% in those who received RT alone. The dire prognosis of those treated without chemoradiation is consistent with other reports [45].

When recurrence occurs within a previously treated RT field, it is not normally possible to retreat with radical doses of radiation due to unacceptable risk of toxicity. Salvage surgery should be considered for pelvic or para-aortic within-field recurrences. Pelvic exenteration is usually the only curative option and can have excellent outcomes. In patients who are unsuitable for or unwilling to undergo surgical salvage, management is with palliative chemotherapy or palliative care. Radiotherapeutic options include palliative radiotherapy or stereotactic radiotherapy.

SBRT

Stereotactic body radiotherapy (SBRT) delivers highly conformal hypofractionated, i.e., short courses of high dose per fraction, doses of radiation with submillimeter accuracy to allow targeting of malignant tissue while sparing adjacent normal structures. Fiducial markers and real-time image guidance are required. It allows delivery of focal areas of high dose within a previously treated pelvis. Although SBRT has not been studied within phase III trials, single institutions have published their experience in cervix cancer. Acceptable toxicity and promising rates of local control in the recurrent setting have been reported [46].

Incurable Disease

If fitness allows, palliative combination chemotherapy is first-line treatment for metastatic disease and is reviewed in another chapter.

Palliative Radiotherapy

Palliative radiotherapy has an established role in incurable cervix cancer and can be used as primary treatment or in the context of metastatic or recurrent disease. In patients with localized disease who are not fit for radical treatment, pelvic radiation (e.g., at doses of 30 Gy in 10 fractions, 20 Gy in 5 fractions, or 8 Gy in a single fraction) can be used to help control vaginal bleeding or achieve a degree of local control [47]. Painful bone metastases can also be treated with similar doses and can achieve improvements in symptoms with response rates up to 60% and complete pain response rates of 34% [48].

Future Directions

Using established cytotoxic agents in addition to standard chemoradiation might be helpful. OUTBACK and INTERLACE are phase III trials currently recruiting patients to establish the role of neoadjuvant (prior to chemoradiation) or adjuvant (following chemoradiation) carboplatin and paclitaxel in improving overall survival. As in other tumor sites, there may be a role for using novel systemic agents to improve radiosensitivity and overcome tumor hypoxia, but as yet, none of these approaches have shown much promise.

RetroEMBRACE data suggests IGABT may obviate the need for concurrent chemotherapy. It also suggests that in carefully selected smaller tumors, dose de-escalation may be safe in terms of disease control with the advantage of minimizing long-term toxicity. EMBRACE2 is an ongoing prospective, multicenter interventional study that aims to improve outcomes by standardizing RT delivered with IMRT and daily image guidance. Stereotactic radiotherapy may be further refined in order to fully exploit the therapeutic window in sites of disease not anatomically amenable to brachytherapy.

Recurrent disease remains difficult to manage and has an unacceptable prognosis. As well as improving primary treatment modalities, there may be opportunities to detect recurrence earlier with improvements in nuclear imaging techniques or a deepening understanding of circulating tumor cells.

In addition to researching optimization of current treatment options, the global healthcare community must work to improve radiotherapy access in the developing world to overcome the unacceptable geographical disparities in mortality. This is especially important given that countries are without primary and secondary prevention measures and therefore a higher burden of disease.

References

1. http://globocan.iarc.fr/old/FactSheets/cancers/cervix-new.asp. 2012.
2. Abdel-Wahab M, Fidarova E, Polo A. Global access to radiotherapy in low- and middle-income countries. Clin Oncol. 2017;29(2):99–104.
3. Colombo N, et al. Cervical cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up ESMO Guidelines Working Group. Ann Oncol. 2012;23(suppl_7):vii27–32.
4. Lax S. Histopathology of cervical precursor lesions and cancer. Acta Dermatovenerol Alp Pannonica Adriat. 2011;20(3):125–33.
5. Cleary C, et al. Biological features of human papillomavirus-related head and neck cancers contributing to improved response. Clin Oncol. 2016;28(7):467–74.
6. Ramirez PT, Jhingran A, Macapiniac HA, et al. Laparoscopic extraperitoneal para-aortic lymphadenectomy in locally advanced cervical cancer: a prospective correlation of surgical findings with positron emission tomography/computed tomography findings. Cancer. 2011;117:1928–34.
7. Moscucci O. The “ineffable freemasonry of sex”: feminist surgeons and the establishment of radiotherapy in early twentieth-century Britain. Bull Hist Med. 2007;81(1):139–63.
8. Withers HR. The four R’s of radiotherapy. Adv Radiat Biol. 1975;5:241–71.
9. Radiotherapy Dose Fractionation Second Edition Gynaecological Cancers Royal College of Radiologists. 2016.

10. Taylor A, Rockall AG, Reznek RH, Powell ME. Mapping pelvic lymph nodes: guidelines for delineation in intensity-modulated radiotherapy. Int J Radiat Oncol Biol Phys. 2005;63(5):1604–12. Epub 2005 Sep 29.
11. Hasselle MD, Rose BS, Kochanski JD, Nath SK, Bafana R, Yashar CM, Hasan Y, Roeske JC, Mundt AJ, Mell LK. Clinical outcomes of intensity-modulated pelvic radiation therapy for carcinoma of the cervix. Int J Radiat Oncol Biol Phys. 2011;80(5):1436–45. https://doi.org/10.1016/j.ijrobp.2010.04.041. Epub 2010 Aug 12.
12. Mundt AJ, Mell LK, Roeske JC. Preliminary analysis of chronic gastrointestinal toxicity in gynecology patients treated with intensity-modulated whole pelvic radiation therapy. Int J Radiat Oncol Biol Phys. 2003;56(5):1354–60.
13. Mundt AJ, Lujan AE, Rotmensch J, Waggoner SE, Yamada SD, Fleming G, Roeske JC. Intensity-modulated whole pelvic radiotherapy in women with gynecologic malignancies. Int J Radiat Oncol Biol Phys. 2002;52(5):1330–7.
14. Meta-analysis Collaboration (CCCMAC). Reducing uncertainties about the effects of chemoradiotherapy for cervical cancer: individual patient data meta-analysis. Chemoradiotherapy for Cervical Cancer Meta-analysis Collaboration (CCCMAC). Cochrane Database Syst Rev. 2010;(1):CD008285. https://doi.org/10.1002/14651858.CD008285. Review.
15. Liu R, Wang X, Tian JH, Yang K, Wang J, Jiang L, Hao XY. High dose rate versus low dose rate intracavity brachytherapy for locally advanced uterine cervix cancer. Cochrane Database Syst Rev. 2014;2014(10):CD007563. https://doi.org/10.1002/14651858.CD007563.pub3.
16. Stewart AJ, Viswanathan AN. Current controversies in high-dose-rate versus low-dose-rate brachytherapy for cervical cancer. Cancer. 2006;107:908–15. https://doi.org/10.1002/cncr.22054.
17. Viswanathan AN, Moughan J, Small W, et al. The quality of cervical cancer brachytherapy implantation and the impact on local recurrence and disease-free survival in RTOG prospective trials 0116 and 0128. Int J Gynecol Cancer. 2012;22(1):123–31. https://doi.org/10.1097/IGC.0b013e31823ae3c9.
18. Potter R, et al. Recommendations from gynaecological (GYN) GEC ESTRO working group (II): concepts and terms in 3D image-based treatment planning in cervix cancer brachytherapy—3D dose volume parameters and aspects of 3D image-based anatomy, radiation physics, radiobiology. Radiother Oncol. 2006 Jan;78(1):67–77.
19. Landoni F, Maneo A, Colombo A, et al. Randomised study of radical surgery versus radiotherapy for stage Ib-IIa cervical cancer. Lancet. 1997;350(9077):535–40.
20. Rotman M, Sedlis A, Piedmonte MR, et al. A phase III randomized trial of postoperative pelvic irradiation in stage IB cervical carcinoma with poor prognostic features: follow-up of a gynecologic oncology group study. Int J Radiat Oncol Biol Phys. 2006;65:169–76.
21. Rogers L, Siu SSN, Luesley D, Bryant A, Dickinson HO. Radiotherapy and chemoradiation after surgery for early cervical cancer. Cochrane Database Syst Rev. 2012;5:CD007583. https://doi.org/10.1002/14651858.CD007583.pub3.
22. Bilek K, Ebeling K, Leitsmann H, Seidel G. Radical pelvic surgery versus radical surgery plus radiotherapy for stage IB carcinoma of the cervix uteri: preliminary results of a prospective randomised clinical study. Archiv fur Geschwulstforschung. 1982;52(3):223–9.
23. Peters WA III, Liu PY, Barrett RJ II, Stock RJ, Monk BJ, Berek JS, Souhami L, Grigsby P, Gordon W Jr, Alberts DS. Concurrent chemotherapy and pelvic radiation therapy compared with pelvic radiation therapy alone as adjuvant therapy after radical surgery in high-risk early-stage cancer of the cervix. J Clin Oncol. 2000;18(8):1606–13.
24. Yamashita H, Okuma K, Kawana K, Nakagawa S, Oda K, Yano T, Kobayashi S, Wakui R, Ohtomo K, Nakagawa K. Am J Clin Oncol. 2010;33(6):583–6.
25. Han K, Milosevic M, Fyles A, et al. Evidence of improved survival surveillance, epidemiology, and end results (SEER) database trends in the utilization of brachytherapy in cervical cancer in the United States. Int J Radiat Oncol Biol Phys. 2013;87:111.
26. Tanderup K, Fokdal LU, Sturdza A, et al. Effect of tumor dose, volume and overall treatment time on local control after radiochemotherapy including MRI guided brachytherapy of locally

advanced cervical cancer. Radiother Oncol. 2016;120(3):441–6. https://doi.org/10.1016/j.radonc.2016.05.014. Epub 2016 Jun 24.
27. MacDonald OK, et al. Prognostic significance of histology and positive lymph node involvement following radical hysterectomy in carcinoma of the cervix. Am J Clin Oncol. 2009;32(4):411–6. https://doi.org/10.1097/COC.0b013e31819142dc.
28. Hoskin PJ, Goh V. Radiotherapy in practice: imaging. Oxford: Oxford University Press; 2010.
29. Vargo JA, et al. Extended field intensity modulated radiation therapy with concomitant boost for lymph node–positive cervical cancer: analysis of regional control and recurrence patterns in the positron emission tomography/computed tomography era. Int J Radiat Oncol Biol Phys. 2014 Dec 1;90(5):1091–8.
30. https://clinicaltrials.gov/ct2/show/NCT01793701
31. Cihoric N, Tapia C, Krüger K, Aebersold DM, Klaeser B, Lössl K. IMRT with 18FDG-PET\CT based simultaneous integrated boost for treatment of nodal positive cervical cancer. Radiat Oncol. 2014;9:83. https://doi.org/10.1186/1748-717X-9-83.
32. Perez CA, Grigsby PW, Castro-Vita H, Lockett MA. Carcinoma of the uterine cervix. Impact of prolongation of overall treatment time and timing of brachytherapy on outcome of radiation therapy. Int J Radiat Oncol Biol Phys. 1995;32(5):1275–88.
33. Song S, Rudra S, Hasselle MD, Dorn PL, Mell LK, Mundt AJ, Yamada SD, Lee NK, Hasan Y. The effect of treatment time in locally advanced cervical cancer in the era of concurrent chemoradiotherapy. Cancer. 2013;119:325–31. https://doi.org/10.1002/cncr.27652.
34. Fyles A, Keane TJ, Barton M, Simm J. The effect of treatment duration in the local control of cervix cancer. J Radiother Oncol. 1992;25(4):273–9.
35. Chen SW, Liang JA, Yang SN, et al. The adverse effect of treatment prolongation in cervical cancer by high-dose-rate intracavitary brachytherapy. Radiother Oncol. 2003;67:69.
36. Evans JC, Per Bergsjfø. The influence of Anemia on the results of radiotherapy in carcinoma of the cervix. Radiology. 1965;84:709–17. https://doi.org/10.1148/84.4.709.
37. Grogan M, Thomas GM, Melamed I, Wong FL, Pearcey RG, Joseph PK, Portelance L, Crook J, Jones KD. The importance of hemoglobin levels during radiotherapy for carcinoma of the cervix. Cancer. 1999;86(8):1528–36.
38. Goubran HA, Elemary M, Radosevich M, Seghatchian J, El-Ekiaby M, Burnouf T. Impact of transfusion on cancer growth and outcome. Cancer Growth Metastasis. 2016;9:1–8. https://doi.org/10.4137/CGM.S32797.
39. Peppone LJ, Mustian KM, Morrow GR, et al. The effect of cigarette smoking on cancer treatment-related side effects. Oncologist. 2011;16(12):1784–92. https://doi.org/10.1634/theoncologist.2011-0169.
40. Fawaz ZS, Barkati M, Beauchemin M-C, Sauthier P, Gauthier P, Nguyen TV. Cervical necrosis after chemoradiation for cervical cancer: case series and literature review. Radiat Oncol (Lond Eng). 2013;8:220. https://doi.org/10.1186/1748-717X-8-220.
41. Gadducci A, Tana R, Cosio S, Cionini L. Treatment options in recurrent cervical cancer (review). Oncol Lett. 2010;1(1):3–11. https://doi.org/10.3892/ol_00000001.
42. Friedlander M, Grogan M. Guidelines for the treatment of recurrent and metastatic cervical cancer. U.S. Preventative Services Task Force. Oncologist. 2002;7(4):342–7.
43. Jain P, Hunter RD, Livsey JE, Coyle C, Swindell R, Davidson SE. Salvaging locoregional recurrence with radiotherapy after surgery in early cervical cancer. Clin Oncol (R Coll Radiol). 2007;19(10):763–8.
44. Chou HH, Wang CC, Lai CH, Hong JH, Ng KK, Chang TC, Tseng CJ, Tsai CS, Chang JT. Isolated paraaortic lymph node recurrence after definitive irradiation for cervical carcinoma. Int J Radiat Oncol Biol Phys. 2001;51:442–8.
45. Grigsby PW, Vest ML, Perez CA. Recurrent carcinoma of the cervix exclusively in the paraaortic nodes following radiation therapy. Int J Radiat Oncol Biol Phys. 1994;28:451–5.
46. Park HJ, et al. Stereotactic body radiotherapy for recurrent or oligometastatic uterine cervix Cancer: a cooperative study of the Korean Radiation Oncology Group (KROG 14-11). Anticancer Res. 2015;35(9):5103–10.

47. Van Lonkhuijzen L, Thomas G. Palliative radiotherapy for cervical carcinoma, a systematic review. 2011. In: Database of abstracts of reviews of effects (DARE): quality-assessed reviews [internet]. York: Center for Reviews and Dissemination; 1995.
48. Sze WM, Shelley MD, Held I, et al. Palliation of metastatic bone pain: single fraction versus multifraction radiotherapy. A systematic review of randomised trials. 2003. In: Database of abstracts of reviews of effects (DARE): quality-assessed reviews [internet]. York: Center for Reviews and Dissemination; 1995.

Quality of Life in Women with Cervical Cancer

15

C. Rutherford, R. Mercieca-Bebber, M. Tait, Linda Mileshkin, and M. T. King

A cervical cancer diagnosis and its treatments can affect health-related quality of life (HRQOL) in many ways, in both the short and long term. HRQOL may be assessed in the context of research or clinical practice and, if used to its full advantage, can be a key component of patient-centred care. HRQOL information from clinical studies can complement clinical data to guide improvements in clinical practice and to counsel individual patients about the impact of treatment, assisting them with treatment decisions. Use of individual patient HRQOL data in clinic has been proven to facilitate doctor-patient communication about issues impacting patient quality of life, helping clinicians to identify and manage patient problems. In this chapter, we introduce terminology and discuss how cervical cancer and its various treatments affect patients' HRQOL including side effects of treatment and physical, psychological, and sexual function issues.

C. Rutherford (✉) · M. Tait
Faculty of Science, School of Psychology, University of Sydney, Sydney, Australia
e-mail: claudia.rutherford@sydney.edu.au; ann.tait@sydney.edu.au

R. Mercieca-Bebber · M. T. King
Faculty of Science, School of Psychology, University of Sydney, Sydney, Australia

Faculty of Medicine, Sydney Medical School, Central Clinical School, University of Sydney, Sydney, Australia
e-mail: rebecca.mercieca@sydney.edu.au; madeleine.king@sydney.edu.au

L. Mileshkin
Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia
e-mail: linda.mileshkin@petermac.org

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8_15

Terminology and Definitions: HRQOL and PROs

A widely accepted definition of *health-related quality of life* (HRQOL) that is useful for clinical trials and health services research is:

> Health-related quality of life (HRQOL) is a multidimensional construct encompassing perceptions of both positive and negative aspects of dimensions, such as physical, emotional, social, and cognitive functions, as well as the negative aspects of somatic discomfort and other symptoms produced by a disease or its treatment. [1]

Integral to this definition is that HRQOL is *multidimensional*, including core domains plus symptoms that will differ across diseases and treatments. It is a *subjective* phenomenon, so the patient's assessment is preferred to that of a proxy such as a relative or attending nurse or doctor [1, 2].

As well as functioning and symptoms, there are many other important aspects of a person's experience of disease and treatment that may have a direct impact on HRQOL, such as satisfaction with care, unmet needs for information or support services, and psychological adjustment to illness. Another term closely related to HRQOL is *patient-reported outcome*. This term emerged to solve the difficulty of finding a universal definition for HRQOL and related concepts. A PRO is defined as:

> A patient-reported outcome (PRO) is any report of the status of a patient's health condition that comes directly from the patient, without interpretation of the patient's response by a clinician or anyone else. [3]

The term PRO does not tell us what is being measured, only that the patient is providing the data. PROs can be symptoms (e.g. pain, anxiety, nausea, fatigue), aspects of functioning (e.g. physical, emotional, sexual, social), and multidimensional constructs (e.g. HRQOL). For the purpose of this chapter, the PRO of interest is HRQOL.

How Cervical Cancer Affects HRQOL

Cervical cancer and its treatments can affect HRQOL in many ways, both positively and negatively, from diagnosis through to acute treatment and survivorship phases. During these various phases, treatments may be preventative, curative, or palliative. There will be differences in symptoms and side effects [4] and differences during the acute treatment and survivorship phases. Patients will also differ in which HRQOL outcomes they are willing to trade off for specific treatment benefits (symptom palliation or increased chance of survival). Patients value benefits and harms of treatment differently and vary in how much risk, loss, regret, or challenge to their personal life they prefer.

Some patients may experience signs and symptoms of the cancer before diagnosis, such as vaginal bleeding between periods, menstrual bleeding that is longer or heavier than usual, bleeding after intercourse and/or menopause, pain during

intercourse, unusual vaginal discharge, excessive tiredness, leg pain or swelling, and lower back pain [5]. Urinary incontinence may also be apparent before treatment: one study reported 29% of patients experienced stress incontinence and 8% urgency incontinence before treatment [6], but not all women will be symptomatic at diagnosis.

A diagnosis of cervical cancer will often have a major impact on a woman emotionally, causing fear and anxiety [7]. These women can experience psychological distress due to potential issues with physical and sexual function, body image, sense of femininity, and fertility. Asymptomatic and younger women often experience shock at the diagnosis and struggle to come to terms with the possibility of surgery or radiotherapy treatment leading to infertility. Additional distress often follows surgical or chemoradiation treatments as these may cause treatment-related effects including urinary, gastrointestinal, and neurologic side effects, physical changes, and sexual dysfunction. Some side effects and changes are chronic, such as psychosexual problems or bowel dysfunction after treatment. These physical and psychological disturbances may adversely affect the ability of women to perform their usual roles and activities. For some women, these adverse impacts are short-lived, returning to previous levels of functioning after a few months [8].

Surgery to excise the cancer may be followed by adjuvant therapy to decrease the chance of the cancer returning or spreading. This proves curative for some patients and prolongs survival for others. If the disease spreads to other tissues (metastases), palliative therapy is intended to reduce disease activity and symptoms. While palliative therapy may extend survival, it may also cause toxicity, so this is a context where PROs are particularly important and could be included as primary endpoints in clinical trials [9].

Proximal Versus Distal Effects on HRQOL

Figure 15.1 illustrates how cervical cancer and its treatments may affect a person's HRQOL. Proximal effects occur directly as a consequence of the cervical cancer and/or treatment for the disease, such as symptoms of the cancer itself (e.g. pain, fatigue) and side effects and toxicities from treatment (e.g. nausea, vomiting) [10]. These may consequently affect a person's ability to function and their overall sense of wellbeing, i.e. cause distal effects. A cervical cancer diagnosis, recurrence, and treatment can directly (i.e. proximally) impact psychological wellbeing or indirectly impact via experience of symptoms, side effects, and loss of functional ability and infertility.

The proximal/distal distinction is important when choosing a PRO instrument to use in cervical cancer because distal outcomes will be influenced by factors external to healthcare, such that the effects of treatment will be increasingly smaller, the more distal the PRO measure becomes [10]. Therefore, a proximal outcome is more likely to be more sensitive to treatment effects than a distal measure and therefore appropriate as the sole or key PRO.

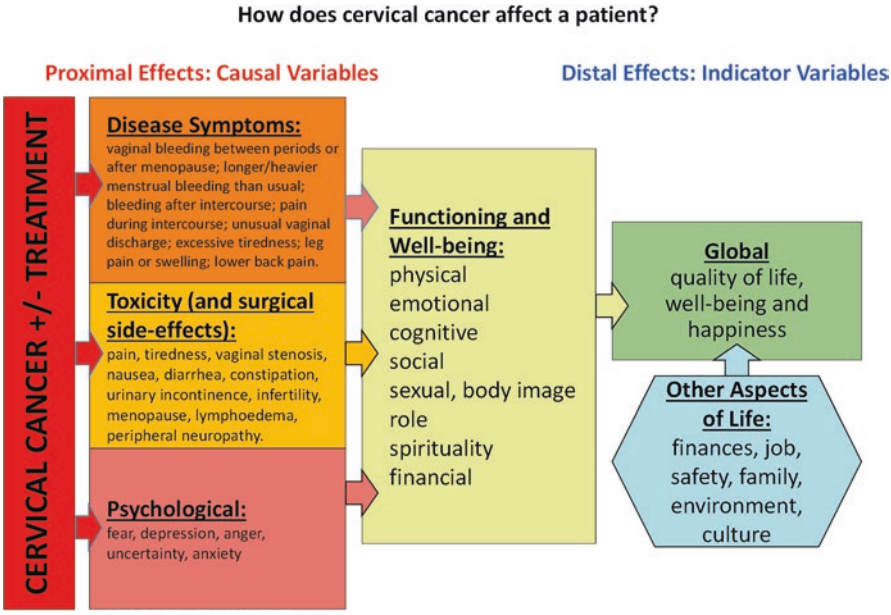

Fig. 15.1 Cervical cancer effects on HRQOL

Treatment-Specific HRQOL: Physical Function and Symptoms and Side Effects of Treatment

A diagnosis of cervical cancer may lead to surgical treatment and/or the use of chemoradiation. Despite life-preserving benefits, treatments for cervical cancer are not without cost, and patient reports of treatment effects may be a useful adjunct to clinician ratings of adverse event criteria and may even be more reliable [11].

Acute, chronic, and delayed treatment toxicities and other side effects can be more than just minor inconveniences. Patients may experience considerable dysfunction due to their treatment, with adverse effects to their HRQOL during and after completion of treatment. Eighty to ninety percent of patients treated for cervical cancer experience pain, fatigue, nausea and vomiting, loss of appetite or weight, anorexia/cachexia, and difficulty sleeping [12]. Some effects may persist long term, while others will return to pre-disease and pretreatment levels. Both the number and severity of symptoms contribute to overall symptom burden, and greater symptom burden tends to be associated with a greater reduction in HRQOL [4]. During treatment, patients may experience side effects that can hinder HRQOL and make it difficult for some to complete treatment. When asked about the late effects of treatment, cervical cancer survivors reported incontinence to both urine and faeces, with some patients carrying diapers in their bag and needing to orient themselves with the nearest toilet [13]. Others avoided large gatherings to avoid exacerbating their tinnitus. Chronic pelvic pain (persistent pain in hips, groins, or lower back) is poorly

described in studies of cervical cancer survivors; however, pelvic, bladder, and bowel pain can restrict everyday life [13].

Surgery

Surgery is the most common treatment for cervical cancer [14]. Surgical procedures vary and can have a differential impact on HRQOL depending on the invasiveness of the surgery and surgical technique [15, 16]. The extent of the cervical cancer will determine the type of surgery received and, in women with less invasive cervical cancer, whether fertility can be preserved [14].

Radical trachelectomy is suitable for women with early-stage, invasive cervical cancers and has been associated with good survival outcomes for women who do not have evidence of pelvic lymph node metastasis [17]. This technique may be performed using different approaches, including radical vaginal trachelectomy (performed both laparoscopically and transvaginally in women with lesions <2 cm in diameter) or abdominal radical trachelectomy (possible for women with larger lesions) [17]. Although intended to preserve fertility (so it may still be possible to become pregnant), complications of radical trachelectomy include chronic vaginal discharge, abnormal uterine bleeding, dysmenorrhoea, inflammation and ulcer due to cerclage, amenorrhoea, cervical stenosis, and pregnancy complications including mid-trimester miscarriage and premature labour [18].

Cervical conisation (or large loop excision of the transformation zone) may be offered to women diagnosed with cervical intraepithelial neoplasia 3 (a precursor lesion of squamous cell carcinoma of the cervix uteri). This procedure involves removal of the lesion and transformation zone and may result in pain, scarring, and increased risk of miscarriages and premature births due to weakening of the cervix [19, 20]. The ongoing Evaluation of Clinical Outcome after Reduction of Conization Size trial (German Clinical Trials Register (DRKS) Identifier: DRKS00006169) is testing whether minimising the cone dimension (resection of the lesion only) reduces the negative risks associated with preterm delivery in women with cervical intraepithelial neoplasia 3 as compared to regular cervical conisation [20]. However, no PROs are being collected.

Women with more invasive disease require more extensive surgery: hysterectomy. Radical hysterectomy (RH) is the most extensive, involving removal of the uterus and supporting ligaments, the cervix and part of the vagina around the cervix. In premenopausal women under 40 years, the surgeon may choose not to remove one ovary to prevent early onset of menopause; however it is not possible for these women to become pregnant due to removal of the uterus (*the impact of loss of fertility and early-onset menopause on women is described in the section on Psychological Impact*). RH may damage pelvic autonomic nerves leading to various sexual and urinary function issues [21], which may lead to problems with emotional and social function – although more PRO evidence is needed to support this hypothesis. Some of the physical changes such as mood swings, hot flushes, and lack of energy that occur following a hysterectomy are consistent with entering menopause [22].

A simple hysterectomy is less extensive than RH, involving removal of the cervix, womb, a small portion of the upper vagina, and the lymph nodes around the womb. The ongoing SHAPE trial (CRUK/13/015) is comparing radical versus simple hysterectomy in early-stage disease, to determine whether this less extensive approach is as effective as RH in treating cervical cancer and whether it confers any benefits in terms of fewer side effects and better HRQOL [23].

Since the 1960s, nerve-sparing techniques have been used in an attempt to preserve bladder, bowel, and sexual functions to some extent [24]. A systematic review comparing RH to nerve-sparing radical hysterectomy (NSRH) found better HRQOL for patients in the NSRH group [25]. A meta-analysis of PROs was not possible due to variation in the methods, instruments used, and reporting across studies [25]. A second systematic review and meta-analysis comparing NSRH to RH determined that NSRH was associated with significantly lower risk of urinary incontinence, shorter mean time to catheterisation, and better sexual function [26]. All of these outcomes (although not all patient-reported) will have an impact on HRQOL. Urinary incontinence may affect psychosocial outcomes, self-confidence, sexual and physical function, and social participation [27]. Likewise, catheters are associated with morbidities including urinary tract infections and pain [28].

Lower urinary tract dysfunction (LUTD), the most common complication after RH, can significantly impair HRQOL [29]. Patients reported abnormal bladder sensation/dysfunction (0–60%), dysuria (0–55%), incontinence (0–100%), urinary retention (10–25%), urinary symptoms such as urgency and frequency (4–75%), constipation (4–31%), and diarrhoea (3–29%) following a RH [25]. Urinary function was better in the NSRH patients. A study comparing concurrent chemoradiation and RH did not find a significant difference in LUTD [30]. Voiding dysfunction was significantly higher in RH, while storage dysfunction, particularly low bladder compliance and increased bladder sensation, was significantly more prevalent in the chemoradiation group; urinary incontinence was not significantly different between groups [30]. Another study found significant deterioration in anorectal functions (e.g. defecation straining, defecation regularity, frequency of defecation) following RH, particularly bladder functions; 53% of women were straining to void when urinating 6 months post-RH [15]. It seems that NSRH improves HRQOL by selectively sparing innervation of the bladder, bowel, and vagina, reducing treatment-induced morbidity, particularly bladder function [25].

Body image post-surgery has been shown to improve over time [31]. No difference was identified in the sexual satisfaction of women who received fertility-sparing surgery compared to those who had RH [32]. However, the combination of minimally invasive (e.g. performed with laparoscope) and nerve-sparing procedures appears to be associated with better sexual function. For example, a study comparing laparoscopic NSRH to standard laparoscopic RH determined that both techniques were associated with reduced sexual functioning but less so in the laparoscopic NSRH group [16]. NSRH and RH are similar in terms of local and overall rate of recurrence and constipation [26] and 2-, 3-, and 5-year survival [25]. Collectively, these findings demonstrate the value of PROs for revealing important information

about the impact of surgical options on women treated for cervical cancer despite potentially similar survival and recurrence outcomes.

Radiotherapy or Radiation

Many women with cervical cancer experience acute side effects either during or immediately after radiotherapy. Although some acute effects are common, most women recover from acute toxicities over a few months, with only some toxicities becoming chronic in a small percentage of patients. Some women may also experience mild or severe late radiation effects such as chronic bowel, urinary, and sexual problems [33], which can have a distal effect on HRQOL outcomes [34]. In some but not all reports, toxicity appears more pronounced in older patients [35].

Late effects of radiotherapy include gastrointestinal, urological, female reproductive tract, skeletal, and vascular toxicity, pain, and development of lymphoedema or secondary malignancies, all with resultant distal HRQOL impacts. A review synthesising HRQOL data from long-term follow-up studies of cervical cancer patients found that patients who receive radiotherapy have the highest risk of increased long-term dysfunction of the bladder and bowel, sexual dysfunction, and psychosocial consequences [36]. One study found that patients who had radiotherapy after laparoscopic surgical staging (group 1) more commonly experienced lower extremity oedema (LEE) and related symptoms than patients who had primary radiotherapy (group 2) [37]. Incidence and duration of LEE were significantly longer in group 1. Nearly 10 years after completing treatment, 48% of patients in group 1 were clinically diagnosed with lymphoedema compared with no patients in group 2. General swelling, limb swelling, and heaviness were significantly higher in group 1. One patient in group 1 developed lymphoedema-related angiosarcoma 7.8 years after surgery. Another study of 91 cervical cancer survivors surveyed >5 years post-radiotherapy found pain in lower back and hips was significantly more prevalent in women who had cervical cancer compared to the general female population, suggesting pain may be due to late effects of radiation [38]. These women had significantly lower HRQOL, higher levels of anxiety and depression, impaired sleep and concentration, and more bladder and intestinal problems than those without chronic pelvic pain.

The worst HRQOL and toxicity outcomes are seen in women who have surgery followed by chemoradiation [30, 39]. Surgery therefore tends to be offered to women who appear to have sufficiently early-/low-risk disease that is not likely to lead to a recommendation for post-operative chemoradiation [14]. Chemoradiation studies that have assessed or reported PROs suggest a transient impact of chemoradiation, where several treatment-related symptoms and problems may develop and persist, either immediately or gradually after treatment. A review of the acute and long-term toxicity of radiotherapy given with or without chemotherapy for cervical cancer found acute toxicity (all grades) of radiotherapy reported in 61% of patients in the rectosigmoid, 27% as urological, 27% as skin, and 20% as gynaecological toxicity [40]. Moderate and severe morbidity consists of 5–7% gastrointestinal and

1–4% genitourinary toxicity. Adding chemotherapy to radiotherapy increases acute haematological toxicity, nausea and vomiting, and fatigue in up to 37% of patients.

Fatigue is a common symptom following treatment for cervical cancer. Younger women report fatigue more frequently than older women, and fatigued women report more anxiety and depression and poorer HRQOL [41]. In younger women treated with radiotherapy, impacts on HRQOL can also be seen both acutely and chronically as a result of the induction of premature menopause with treatment. Radiation therapy for cervical cancer can directly damage ovarian follicles, leading to early menopause. Menopausal symptoms can include vaginal dryness, hot flushes, and mood changes [22]. Longer-term issues can include osteoporosis, decreased libido or other sexual side effects, and weight gain. Ovarian failure can occur immediately after treatment depending on the patient's age and the dose of fractionated radiotherapy [42]. A systematic review on ovarian transposition in gynaecological cancer (93% cervical cancer) found that surgically repositioning the ovaries can preserve ovarian function in 94% of women having brachytherapy and 65% of women undergoing both brachytherapy and external beam radiotherapy [43]. Gynaecological toxicity usually occurs shortly after treatment and may result in vaginal dryness or bleeding as well as shortening and contraction of the vagina resulting in impacts on sexual function.

One longitudinal study of 744 patients with locally advanced cervical cancer who had definitive chemoradiation with image-guided adaptive brachytherapy found general HRQOL and emotional and social functioning were impaired at baseline relative to age- and sex-matched population norms but improved during the first 6 months after treatment to population norm levels, whereas cognitive functioning remained impaired [39]. The causes of these baseline HRQOL deficits are unclear but may be related to socioeconomic disadvantage among patients with cervical cancer [44, 45]. The lowest baseline scores were seen in social and role functioning but increased after treatment to reach a plateau at 6 months and then declined slightly at 3 and 4 years. Tumour-related symptoms present before treatment (e.g. pain, appetite loss, and constipation) also decreased substantially 3 months after treatment [39]. Several treatment-related symptoms (diarrhoea, menopausal symptoms, peripheral neuropathy, and sexual functioning problems) developed immediately after treatment and persisted over time, while lymphoedema and dyspnoea developed gradually after treatment.

For at least 20 years after chemoradiation treatment, new side effects may develop. Gastrointestinal toxicity usually occurs in the first 2 years after treatment in about 10% of patients and may manifest as persistent bowel urgency, diarrhoea, incontinence, or rectal bleeding [40]. The incidence of moderate and severe urological toxicity can increase up to 10% and rises over time and may manifest as urinary urgency, incontinence, or bleeding [40]. In more severe cases, fistulas between various parts of the bowel, bladder, and/or vagina may occur. Skeletal and vascular toxicity, including the development of pelvic insufficiency fractures, can occur years to decades later. At present, no increase in late toxicity has been observed after the addition of cisplatin to radiotherapy. The review of studies of radiotherapy given with or without chemotherapy for cervical cancer notes that most randomised

studies in cervical cancer have a limited follow-up period [40]; therefore evidence about longer-term treatment-related side effects and HRQOL impacts is lacking.

Chemotherapy

Chemotherapy is the standard treatment for locally advanced or metastatic cervical cancer [5]. Due to its systemic nature, chemotherapy tends to present with similar side effects across cancer types. Hair loss (alopecia), the most common side effect of many chemotherapies, reduces self-confidence, self-esteem, and body image [46]. These effects do not always disappear with regrowth of hair and in some cases may negatively impact sexual functioning and relationships. Despite these impacts, examination of the effects of chemotherapy-induced alopecia on more general HRQOL has been limited to descriptive studies rather than controlled designs. As there are currently no PRO measures of chemotherapy-induced alopecia, higher-level evidence in this area awaits the advent of a suitable PRO instrument [47].

Chemotherapy-induced nausea and vomiting is currently regarded as one of the most debilitating symptoms, despite guidelines suggesting that it may be prevented in as many as 70% of cases with appropriate antiemetics [48]. Nausea and vomiting is associated with loss of physical, cognitive, and social functioning, global HRQOL, fatigue, anorexia, insomnia, and dyspnoea [49]. Patients with uncontrolled symptoms are more likely to suffer from depression and fatigue [49]. Potential long-term toxicities such as ototoxicity or neurotoxicity manifest as hearing deficits or peripheral neuropathy, which can affect physical function and result in long-term HRQOL impacts.

Cancer and treatment-related neurocognitive dysfunction (CRND), that is, impairments in cognition including attention and memory, information processing speed, and executive functioning, can negatively affect a patient's participation in daily activities and overall HRQOL. No studies have been published directly investigating cognitive function in cervical cancer survivors with neurocognitive tests. Some studies that used the QLQ-C30 questionnaire, which has two items for cognitive function, have found that cervical cancer patients receiving chemoradiation treatment experienced impaired cognitive functioning at specific time points after treatment (3 months and again at 30–36 months) when compared to age-matched females [39]. However, studies have not found differences in cognitive functioning between assessment time points, including baseline, suggesting that cognitive functioning may be affected by a combination of cancer- and treatment-related side effects [39]. Most research into cognitive effects of chemotherapy has been performed in breast cancer. While awareness of these potential effects has increased in recent years, there remain important limitations in our understanding of the mechanisms underlying these changes [50].

Newer Therapies

In 2014, a US-led randomised controlled trial, GOG240, evaluated the benefits of chemotherapy with or without bevacizumab, an anti-angiogenic drug, for advanced cervical cancer [51]. They found that chemotherapy plus bevacizumab improved overall survival (3.7 months) compared to chemotherapy alone but was also linked with higher incidence of fistula (15%) versus only 1% in the chemotherapy-alone group [51]. A sub-analysis of the PROs from GOG240 did not find any significant differences in toxicity or HRQOL between the groups [52].

Cervical Cancer in Low- and Middle-Income Countries

The majority of cervical cancer patients in the developing world present with advanced-stage disease with limited access to adequate standard treatment. A recent analysis of radiation therapy infrastructure in 139 low- and middle-income countries found that only 4 of 139 countries have the requisite number of teletherapy units to manage the estimated burden of cancer in 2020 and 55 (39.5%) have no radiation facilities at present [53]. As a result, the mortality and morbidity rates from cervical cancer are high for women in the developing world. Cervical cancer remains the fourth leading cause of cancer death in women worldwide [54] and is the leading cause of cancer death in women with HIV in sub-Saharan Africa [55]. The impacts of cervical cancer on HRQOL in these settings are likely to be higher than in the developed world, and impaired HRQOL at baseline before treatment may be related to socioeconomic disadvantage, but this topic has not been well studied.

Psychological Impact

The psychological impact of a cervical cancer diagnosis and subsequent treatment can have wide-ranging, long-term impacts on patients' mental health and wellbeing. Women diagnosed with and treated for cervical cancer can experience psychological distress due to reductions in physical functioning, body image, sexual function, and fertility and experience significant fear of cancer recurrence or development of a new cancer [13]. Additional distress often follows surgical, radiotherapy, and chemotherapy treatments as these often cause treatment-related effects including pain, urinary and gastrointestinal side effects, physical changes, and sexual dysfunction. Some side effects and changes are chronic and can significantly affect long-term HRQOL such as psychosexual problems after treatment. Psychological distress may persist long after treatment completion; about 30% of cancer patients suffer from clinically significant psychological distress [56], and up to 97% of cancer survivors report some degree of fear of cancer recurrence [57].

A longitudinal study of 60 women with cervical cancer found that patients experienced significantly higher anxiety prior to surgery than patients prior to

radiotherapy, but the intensity of anxiety gradually decreased in both groups by 6 months post-treatment [58]. Presence of pain and irregular menstrual bleeding are important risk factors for the development of anxiety. Another longitudinal study of 92 patients with precancerous lesions, 93 with early cervical cancer, and 35 with advanced cervical cancer found women with precancerous lesions and early cervical cancer had better psychological wellbeing than women with advanced cervical cancer and those with early cancer recover more quickly than those with advanced disease [7]. A cross-sectional study of 282 patients treated with radical hysterectomy and pelvic lymph node dissection, surgery and adjuvant radiotherapy, or primary radiotherapy found lower mental (and physical) wellbeing and increased body image disturbance associated with severe defecation symptoms [59].

Fertility and Early Menopause

Life-changing consequences of treatment for cervical cancer include infertility, accelerated onset of menopause, and changes in sexual functioning. These can have major psychological impacts. Through surgery, reproductive organs may be removed. Administration of chemotherapy or radiation to the ovary can cause sterility (permanent ovarian failure) in high doses. Radiotherapy will affect the womb so that it is not possible to have children afterwards. Radiotherapy and some chemotherapy drugs can also affect the ovaries bringing on early menopause.

For those where fertility-sparing options are not possible, losing fertility can be very difficult to cope with particularly if a woman had wanted to have a child. Women who lose their fertility as a result of treatment for cervical cancer report feelings of depression, grief, stress, and sexual dysfunction [60]. These women have a multitude of emotions to deal with on top of having to cope with a cancer diagnosis and subsequent recovery following treatment. It is not uncommon for a woman to cope with recovery from surgery while also experiencing cumulative side effects of chemotherapy and/or radiation therapy and hormonal disruption and reproductive failure following surgery. These side effects can have distal effects where cervical cancer survivors may also experience impairments in physical function, bowel and bladder changes, emotional issues, and sexual morbidity. Studies show that some women are unable to process all information given at the time of diagnosis or to think about possible treatment impacts or long-term consequences such as infertility [61, 62].

Psychosexual/Sexual Function

An important aspect of women's HRQOL is their sexual function. One study found 33% of cervical cancer survivors reported sexual distress due to vaginal sexual complaints, body image concerns, sexual enjoyment, and partner's sexual dissatisfaction [63]. Sexual dysfunction in women with cervical cancer is commonly due to physical or psychological impacts caused by the cancer itself or complications of

hysterectomy or radiotherapy that result in vaginal dryness, dyspareunia, vaginal constriction post-radiotherapy, and decreased sexual desire [64–66]. Other physical (e.g. fatigue, vaginal bleeding, and bladder and bowel dysfunction) and psychological (e.g. decreased spontaneity and reduced mood) problems can prevent or interfere with a woman's desire or ability to have sex [13]. A woman's sexuality and self-identity may also be a contributing factor. Some women may be glad to be rid of the cancer and not feel a sense of loss, while others may mourn the loss of their uterus and fertility and experience gender identity and femininity issues [67]. How one perceives oneself may be predictive of future psychological wellbeing. A limitation of the evidence is that many studies that assessed sexual function in women with cervical cancer used non-validated PRO instruments and potential confounders were not considered [66].

Cervical cancer can impact on relationships and place couples at a 40% increased chance of divorce [68]. A cross-sectional survey of 26 couples where the woman had invasive cervical cancer stages I–IV, up to 2 years post-treatment, found both women and their male partners expressed equal intensities of concern regarding the illness and its treatment, sexuality, prognosis, and communication with the treatment team [69]. Couples where the women had advanced cancer expressed higher concerns than those with earlier-stage disease. Although women with cervical cancer reported more fatigue and illness intrusiveness than their male partners, both experienced disruptions in relationships and intimacy [69].

Supportive Care Needs of Cervical Cancer Survivors

A review synthesising the supportive care needs of cervical cancer survivors classified individual needs into ten domains; interpersonal/intimacy (83%), health system/information (67%), psychological/emotional (58%), and physical needs (50%) were needs most frequently explored. Dealing with fear of cancer recurrence, concerns about appearance/body image, lack of sexual desire, requiring more sexuality-related information, managing pain, and dealing with difficulties in relationship with partner were the most frequently cited individual needs (≥4 studies) [70]. However, study limitations precluded drawing conclusions as to how these needs evolve over time from diagnosis to treatment and subsequent survivorship, and whether demographic or clinical variables such as age, race/ethnicity, disease stage, or treatment modality play a moderating role needs future exploration [70]. Another systematic review of HRQOL after treatment in cervical cancer survivors indicated that depression generally increased with age, while anxiety decreased, and radiotherapy was associated with worse long-term HRQOL and sexual function [71].

Women diagnosed with and treated for cervical cancer may benefit from referral to fertility and sexual health counsellors. Reduced levels of distress and regret have been reported in women who receive counselling and the option of fertility-sparing treatment [61, 62]. Menopause clinics may recommend the use of vaginal dilators post-radiotherapy and/or oestrogen cream in an effort to keep the vagina open post-radiotherapy. Of note, some women may have sexual health issues that predate a

diagnosis of cervical cancer such as a history of sexual abuse that leads to not attending for screening pap smears [72] or HPV infection causing the cancer [73].

Exercise and health-promoting behaviours intended to reduce stress and improve psychological wellbeing such as yoga or meditation may be beneficial in the physical and psychological recovery of women treated for cervical cancer. However, while exercise may lower the risk of cervical cancer [74], unlike other areas of oncology where the benefits of exercise for physical and psychological functioning and overall HRQOL are known [75, 76], adequate data is lacking about the benefits of exercise for women with cervical cancer.

The Need for Evaluating HRQOL in Patients with Cancer

The benefits and harms of cervical cancer treatments provide compelling arguments for incorporating the quality of patients' lives into decisions about treatment. Support for this notion has been expressed by clinical trial groups, cancer institutes, drug regulatory bodies, and the pharmaceutical industry [3, 77–83] (Table 15.1).

Methods of Assessing HRQOL in Cervical Cancer

A simple way of assessing HRQOL would be to ask a patient how they are feeling. However, this would likely yield very unreliable results as it would be prone to variations in both the way the question was asked and how the patient responded. A more standardised approach is needed. We do this by asking standard questions about relevant issues with a standard set of response options, in the form of a questionnaire. The questionnaire, along with the algorithm for scoring responses into summary scores for analysis and reporting, is referred to as a PRO instrument or measure.

Table 15.1 Reasons for assessing HRQOL in cervical cancer clinical trials and clinical practice

Baseline HRQOL serves as an independent prognostic factor for survival and locoregional control
In some cases, HRQOL and other PROs may be more sensitive and/or responsive to treatment effects than clinical measures of toxicity
HRQOL data may provide clinicians useful information when communicating with patients about their expectations and assist the patient and clinician in treatment decision-making through better understanding of treatment benefits and risks during the acute and survivorship phases (e.g. impact of chronic side effects)
Information about potential impacts on HRQOL may be one of the factors that patients consider when making decisions about treatments with their clinician and helps patients make informed decisions based on what others have experienced (i.e. likely treatment effects)
PROs can be used to help identify those patients who might benefit from psychosocial interventions

Data from Refs. [2, 9, 81–83]

PRO instruments draw on the psychometric tradition and measure complex variables broken down into their component parts. Each question (item) may ask about a specific issue, for example, "do you have trouble walking?"; this is referred to as the "item stem". The stem will have a rating scale attached, known as "response options". The response options are usually in the form of a Likert scale, i.e. where 0 = not at all and 5 = very much so, enabling us to quantify the patients' response by attaching a numerical value to increasing levels of severity or bother. An item may be grouped with similar items addressing a larger construct, such as physical functioning (often referred to as a domain), to provide a scale score for physical functioning, or the scale may be comprised of only a single item. Any number of domains may be assessed in a single PRO instrument, that is, a PRO instrument may assess only one domain (unidimensional) or several domains (multidimensional).

Patients usually self-complete PRO instruments, in line with the knowledge that HRQOL is subjective and so accordingly the patient should self-interpret each question. This practice also helps to reduce bias that may be introduced if questions are discussed with another individual, in line with the FDA's definition of PROs [3]. However, there are some circumstances where a researcher-administered instrument is necessary, for example, if the patient is fatigued or unable to read or speak the language that the questionnaire is written in. As well as being quick and straightforward to use in research, instruments have the advantage that they yield results that are readily comparable between studies. However, there are always limitations to the information that an instrument, or even a battery of instruments, can provide.

Choosing a PRO Instrument

The large number of available instruments to measure HRQOL and other PROs makes it difficult for researchers to select one, particularly if more than one could be suitable. In brief, researchers should consult clinicians, patients, and the literature to determine which issues are appropriate to the particular research and treatment context. They should then consult databases such as PROQOLID [84], which catalogue a large range of PRO instruments, to identify potentially suitable instruments assessing the domains of importance. These instruments should be reviewed to determine whether the questions address the issues in a meaningful way (i.e. whether they have content and face validity for the research context). The scoring system should be reviewed to determine whether the instrument produces a score for the issue/s of importance to the research study. The literature should also be consulted to determine whether the validation studies were methodologically sound (*refer to the section on what makes a good instrument described in this chapter*) or whether more validation work should be done. Also, consider whether clinically important difference criteria or cut-offs have been established to assist with interpretation of the data. Although established clinically important differences do not exist for common cervical cancer questionnaires, general approaches can be used to interpret PRO scores [85]. A pilot study in the population of interest can be a useful final step to assess the suitability of the instrument.

Key Questions to Consider When Selecting a PRO Instrument

1. Is the intended use for research or in clinical practice?
2. Which issues are important to the particular research and treatment context?
3. Does the PRO instrument cover all the issues that matter in a given context?
4. Does the PRO instrument have evidence for the psychometric properties: validity, reliability, responsiveness, generalisability, and interpretation?
5. Have clinically important difference criteria or cut-offs been established?

What Makes a Good Instrument?

Scientific and methodological rigorous development of a PRO instrument involves careful item selection informed by literature review and expert and patient opinion [3, 86] and testing of the instruments' psychometric properties in populations that the instrument is intended for. Important psychometric properties include validity, reliability, sensitivity, responsiveness, and interpretability. When deciding whether an instrument is "good", consideration of its (1) conceptual and measurement models, (2) validity, (3) reliability, (4) responsiveness to change, (5) interpretability, (6) respondent and administrative burden, (7) alternative forms, and (8) cultural and language adaptations may be a deciding factor. Further, an instrument should be appropriate for the given clinical context, acceptable, and feasible and have precision, should minimise measurement error, and should ensure consistency, ultimately providing a more reliable measurement than what would be obtained by informal interviews. In practice and research, only structured and psychometrically rigorous instruments should be used.

PRO Instruments for Cervical Cancer Clinical Research: Core Cancer Instruments Versus Tumour-Specific Modules

There are many available instruments that measure different PROs. The two most widely used HRQOL instruments in cancer clinical trials are the European Organisation for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire (QLQ-C30 [77]) and the Functional Assessment of Cancer Therapy – General (FACT-G [87]). Both instruments have cervical cancer-specific modules that assess the HRQOL of patients treated for cervical cancer in clinical trials.

EORTC Instruments

The QLQ-C30 is the core instrument of the EORTC's modular approach to HRQOL assessment. It includes HRQOL domains relevant to a range of cancer sites and treatment types. The EORTC conceptualised HRQOL as multidimensional with at least three basic domains: physical functioning, including symptom experience and

functional status; emotional functioning; and social functioning. It has 30 items incorporated into 9 multi-item subscales – 5 functional (physical, role, cognitive, emotional, and social functioning), 3 symptom (fatigue, pain, and nausea/vomiting), and a global health status/HRQOL scale – as well as 6 single items that assess dyspnoea, appetite loss, sleep disturbance, constipation, diarrhoea, and perceived financial impact of disease and treatment. Ratings for each item range from 1 (not at all) to 4 (very much) during the past week. The QLQ-C30 is designed to be used across cancer populations and takes about 11 min to complete [77]. It is available in more than 90 languages. The QLQ-C30 is complemented by modules specific to particular cancers, such as cervical cancer (QLQ-CX24). The core module facilitates comparison of HRQOL across cancers, and the disease-specific modules provide sensitivity for particular trials.

The QLQ-CX24 is the EORTC module specific to cervical cancer. It is a 24-item questionnaire developed in a multicultural and multidisciplinary setting [88]. It incorporates 3 multi-item scales (symptom experience (11 items), body image (3 items), and sexual/vaginal functioning (4 items)) and 6 single-item scales (sexual activity, sexual enjoyment, sexual worry, lymphoedema, peripheral neuropathy, and menopause symptoms). Ratings for each item range from 1 (not at all) to 4 (very much) during the past week. The QLQ-CX24 is meant for all cervical cancer patients across varying disease stages and treatments [88]. It has been translated into 29 languages.

FACIT Instruments

The FACT-G was developed by social scientists over a 5-year period [87]. It is the core component within the Functional Assessment of Chronic Illness Therapy (FACIT) Measurement System.

First trialled in 1992, it has undergone a number of refinements. The current version (version 4; 1997) includes 27 items appropriate for use with patients with any cancer type. Questions represent four primary HRQOL domains: physical wellbeing, social/family wellbeing, emotional wellbeing, and functional wellbeing. As well as domain scores, the instrument yields a total HRQOL score. Ratings for each item range from 0 (not at all) to 4 (very much) during the previous 7 days. The FACT-G is available in 45 languages. In addition to the FACT-G, the FACIT suite includes cancer type (e.g. cervical), treatment (e.g. neurotoxicity from systemic chemotherapy), and symptom-specific (e.g. anorexia/cachexia, fatigue) instruments.

The FACIT approach differs slightly from the EORTC modular system, where stand-alone modules are used in conjunction with the QLQ-C30. In the FACIT system, each of these disease-, treatment-, and symptom-specific instruments implicitly includes the FACT-G instrument. For example, the FACT-Cx instrument contains all 27 questions from the FACT-G plus an additional 15 questions that relate specifically to cervical cancer. The additional 15 items cover urinary issues, appearance/body image, emotional wellbeing, appetite, genital (female), sexual,

constipation, eating/drinking (other than appetite), incontinence (urinary), and reproductive concerns. The FACT-Cx instrument can be self-completed or used in an interview format and takes about 15 min to complete [89]. The FACT-Cx items are rated from 0 (not at all) to 4 (very much) during the previous 7 days.

A number of chemotherapy-specific questionnaires have been designed to assess patients' reports of the side effects that most likely arise from their specific chemotherapy regimens: the Functional Assessment of Cancer Treatment/Gynecologic Oncology Group Neurotoxicity questionnaire (FACT/GOG-Ntx) [90], the FACT-Taxane [91], the Breast Cancer Chemotherapy Questionnaire (BCQ) [92], and the Quality of Life during Cancer Chemotherapy questionnaire (GLQ-8) [93]. These all have evidence of validity and reliability across ovarian, lung, and general cancer patients, respectively; however their use has not yet been evaluated in cervical cancer patients.

Ongoing Clinical Trials in Cervical Cancer

Internationally, there are currently 27 active cervical cancer clinical trials that are collecting PROs. The majority of these studies are coordinated in China, Korea, and the USA, and the treatments investigated are predominately chemoradiation and surgical procedures for women with cervical cancer stages I–IV. PROs being measured include overall HRQOL, treatment toxicity, anorectal symptoms and urinary incontinence, and sexual functioning including psychosexual health. Of the eight trials that list the PRO instruments, six are using the EORTC QLQ-C30 (five also with the QLQ-CX24), two are using the FACT-Cx, one includes a chemotherapy-specific measure (FACT/GOG-Ntx), and five use other measures not specific to cervical cancer (e.g. the EQ-5D; Brief Pain Inventory; Sexual Function-Vaginal Changes questionnaire). Australia is currently leading two trials incorporating PRO assessment: a phase III trial of adjuvant chemotherapy following chemoradiation as primary treatment for locally advanced cervical cancer compared to chemoradiation alone and a phase III randomised clinical trial of laparoscopic or robotic radical hysterectomy versus abdominal radical hysterectomy in patients with early-stage cervical cancer. These trials will provide important patient-reported data about HRQOL and other PROs affected by treatments for cervical cancer.

Conclusions and Recommendations

Surgery, radiotherapy, and chemotherapy are the mainstays of cervical cancer treatment. Many patients receive multiple treatment modalities. Given the high 5-year survival rate, women with cervical cancer constitute a patient population in need for ongoing, person-centred supportive care. PROs can provide valuable information about the benefits and harms of cervical cancer treatments and therefore provide compelling arguments for incorporating the quality of patients' lives into decisions about treatment.

Evaluation and improvements of long-term HRQOL are essential in cervical cancer given that urinary dysfunction and sexual problems are highly prevalent and frequent voiding and diarrhoea may become chronic symptoms. Some symptoms may even develop long after treatment has completed, and there is compelling evidence that symptoms are associated with significant psychological and physical impairment. Therefore, assessment of symptoms and impacts on HRQOL should be considered during patient consultation and post-treatment surveillance to enable better detection and management of symptoms that impact patients' HRQOL. In discussing treatment with their patients, clinicians should consider the benefits and harms treatments might have on their patients' HRQOL in both the short and long term. There is increasing recognition of the psychosexual needs of cervical cancer survivors and the importance of counselling around sexual functioning, fertility, and pregnancy-related complications. Providing supportive care during treatment can reduce the prevalence and magnitude of long-term sequelae of cervical cancer, which will in turn improve psychological outcomes, HRQOL, and the quality of care.

Rigorous studies of long-term PROs by treatment modalities are lacking. There are several approaches to measuring PROs of women with cervical cancer; some are specific to disease stage or treatment and others are general. Suitable choice of instrument(s) should be guided by the research questions, context, and constraints [2, 9, 94]. We have provided a brief overview of the issues to consider when including PROs in cervical cancer clinical trials, such as selecting a suitable instrument [94–96] and reducing missing data [97]. Information about specific instruments can be found on the Mapi Research Trust PROQOLID website. Importantly, HRQOL can successfully complement survival and toxicity endpoints in cervical cancer trials and clinical practice, and provides important, patient-centred information. The International Society of Quality of Life Research has produced guidelines for implementing PRO assessment in clinical practice [98].

Both in clinical research and clinical practice, HRQOL research is a growing field in which evidence is constantly emerging. In this chapter, we provide a brief synthesis of evidence to date and summarise ongoing cervical cancer clinical trials, which will provide evidence for some of the areas of need identified, and current recommendations for incorporating PROs in cervical oncology research and practice. For further information and useful resources, please see websites for the Quality of Life Office, University of Sydney, and the International Society of Quality of Life Research.

References

1. Osoba D. Lessons learned from measuring health-related quality of life in oncology. J Clin Oncol. 1994;12(3):608–16.
2. Revicki DA, Osoba D, Fairclough D, et al. Recommendations on health-related quality of life research to support labeling and promotional claims in the United States. Qual Life Res. 2000;9(8):887–900.

3. Food and Drug Administration. Patient reported outcome measures: use in medical product development to support labelling claims. US Department of Health & Human Support Food & Drug Administration; MD, USA 2009.
4. Deshields TL, Potter P, Olsen S, et al. The persistence of symptom burden: symptom experience and quality of life of cancer patients across one year. Support Care Cancer. 2014;22(4):1089–96.
5. Cancer Council Australia. Understanding cervical cancer: Cancer Council NSW; 2015. https://www.cancer.org.au/content/about_cancer/ebooks/Understanding_Cervical_Cancer_booklet_September_2017.pdf
6. Thomas SG, Sato HR, Glantz JC, et al. Prevalence of symptomatic pelvic floor disorders among gynecologic oncology patients. Obstet Gynecol. 2013;122(5):976–80.
7. Xie Y, Zhao FH, Lu SH, et al. Assessment of quality of life for the patients with cervical cancer at different clinical stages. Chin J Cancer. 2013;32(5):275–82.
8. Costa D, Mercieca-Bebber R, Rutherford C, et al. The impact of cancer on psychological and social outcomes. Aust Psychol. 2016;51:89–99.
9. Au H-J, Ringash J, Brundage M, et al. Added value of health-related quality of life measurement in cancer clinical trials: the experience of the NCIC CTG. Expert Rev Pharm Out. 2010;10(2):119–28.
10. Brenner MH, Curbow B, Legro MW. The proximal-distal continuum of multiple health outcome measures: the case of cataract surgery. Med Care. 1995;33(4):As236–44.
11. Basch E, Jia X, Heller G, et al. Adverse symptom event reporting by patients vs clinicians: relationships with clinical outcomes. J Natl Cancer Inst. 2009;101(23):1624–32.
12. Agarwal S, Bodurka DC. Symptom research in gynecologic oncology: a review of available measurement tools. Gynecol Oncol. 2010;119(2):384–9.
13. Sigaard L, Larsen H, Mikkelsen T, et al. Living experiences with late effects after treatment for cervical cancer. Support Care Cancer. 2015;23(1):S311.
14. Marth C, Landoni F, Mahner S, et al. Cervical cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up†. Ann Oncol. 2017;28(4):iv72–83.
15. Cibula D, Velechovska P, Slama J, et al. Late morbidity following nerve-sparing radical hysterectomy. Gynecol Oncol. 2010;116(3):506–11.
16. Bogani G, Serati M, Nappi R, et al. Nerve-sparing approach reduces sexual dysfunction in patients undergoing laparoscopic radical hysterectomy. J Sex Med. 2014;11(12):3012–20.
17. Arimoto T, Kawana K, Adachi K, et al. Minimization of curative surgery for treatment of early cervical cancer: a review. Jpn J Clin Oncol. 2015;45(7):611–6.
18. Karimi-Zarchi M, Mousavi A, Gilani MM, et al. Conservative treatment in early cervical cancer. Int J Biomed Sci. 2013;9(3):123–8.
19. Arbyn M, Kyrgiou M, Simoens C, et al. Perinatal mortality and other severe adverse pregnancy outcomes associated with treatment of cervical intraepithelial neoplasia: meta-analysis. BMJ. 2008;337:a1284.
20. Schwarz TM, Kolben T, Gallwas J, et al. Comparison of two surgical methods for the treatment of CIN: classical LLETZ (large-loop excision of the transformation zone) versus isolated resection of the colposcopic apparent lesion – study protocol for a randomized controlled trial. Trials. 2015;16:225.
21. Kim HS, Kim K, Ryoo SB, et al. Conventional versus nerve-sparing radical surgery for cervical cancer: a meta-analysis. J Gynecol Oncol. 2015;26(2):100–10.
22. Surgical menopause Victoria, Australia: Australasian Menopause Society Limited; 2013 [cited 2017 August 20]. https://www.menopause.org.au/hp/information-sheets/756-surgical-menopause
23. UK CR. A trial looking at surgery for cervical cancer (SHAPE). Trial number CRUK/13/015. London: Cancer Research UK; 2017 [cited 2017 August 30]. http://www.cancerresearchuk.org/about-cancer/find-a-clinical-trial/a-trial-looking-at-surgery-for-cervical-cancer-shape
24. Maas CP, Trimbos JB, DeRuiter MC, et al. Nerve sparing radical hysterectomy: latest developments and historical perspective. Crit Rev Oncol Hematol. 2003;48(3):271–9.

25. van Gent MD, Romijn LM, van Santen KE, et al. Nerve-sparing radical hysterectomy versus conventional radical hysterectomy in early-stage cervical cancer. A systematic review and meta-analysis of survival and quality of life. Maturitas. 2016;94:30–8.
26. Xue Z, Zhu X, Teng Y. Comparison of nerve-sparing radical hysterectomy and radical hysterectomy: a systematic review and meta-analysis. Cell Physiol Biochem. 2016;38(5):1841–50.
27. Sinclair AJ, Ramsay IN. The psychosocial impact of urinary incontinence in women. Obstet Gynaecol. 2011;13(3):143–8.
28. Hakvoort RA, Thijs SD, Bouwmeester FW, et al. Comparing clean intermittent catheterisation and transurethral indwelling catheterisation for incomplete voiding after vaginal prolapse surgery: a multicentre randomised trial. BJOG. 2011;118(9):1055–60.
29. Aoun F, van Velthoven R. Lower urinary tract dysfunction after nerve-sparing radical hysterectomy. Int Urogynecol J. 2015;26(7):947–57.
30. Katepratoom C, Manchana T, Amornwichet N. Lower urinary tract dysfunction and quality of life in cervical cancer survivors after concurrent chemoradiation versus radical hysterectomy. Int Urogynecol J. 2014;25(1):91–6.
31. Ferrandina G. Long term evaluation of quality of life and emotional distress in patients with cervical cancer. Int J Gynecol Obstet. 2012;119:S188–S9.
32. Chan JL, Letourneau J, Salem W, et al. Sexual satisfaction and quality of life in survivors of localized cervical and ovarian cancers following fertility-sparing surgery. Gynecol Oncol. 2015;139(1):141–7.
33. Korfage IJ, Essink-Bot ML, Mols F, et al. Health-related quality of life in cervical cancer survivors: a population-based survey. Int J Radiat Oncol Biol Phys. 2009;73(5):1501–9.
34. Klee M, Thranov I, Machin PD. The patients' perspective on physical symptoms after radiotherapy for cervical cancer. Gynecol Oncol. 2000;76(1):14–23.
35. Laurentius T, Altendorf-Hofmann A, Camara O, et al. Impact of age on morbidity and outcome of concurrent radiochemotherapy in high-risk FIGO stage I to IVA carcinoma of the uterine cervix following laparoscopic surgery. J Cancer Res Clin Oncol. 2011;137(3):481–8.
36. Pfaendler KS, Wenzel L, Mechanic MB, et al. Cervical cancer survivorship: long-term quality of life and social support. Clin Ther. 2015;37(1):39–48.
37. Kim SI, Lim MC, Lee JS, et al. Comparison of lower extremity edema in locally advanced cervical cancer: pretreatment laparoscopic surgical staging with tailored radiotherapy versus primary radiotherapy. Ann Surg Oncol. 2016;23(1):203–10.
38. Vistad I, Cvancarova M, Kristensen GB, et al. A study of chronic pelvic pain after radiotherapy in survivors of locally advanced cervical cancer. J Cancer Surviv. 2011;5(2):208–16.
39. Kirchheiner K, Potter R, Tanderup K, et al. Health-related quality of life in locally advanced cervical cancer patients after definitive chemoradiation therapy including image guided adaptive brachytherapy: an analysis from the EMBRACE study. Int J Radiat Oncol Biol Phys. 2016;94(5):1088–98.
40. Maduro JH, Pras E, Willemse PH, et al. Acute and long-term toxicity following radiotherapy alone or in combination with chemotherapy for locally advanced cervical cancer. Cancer Treat Rev. 2003;29(6):471–88.
41. Sekse RJ, Hufthammer KO, Vika ME. Fatigue and quality of life in women treated for various types of gynaecological cancers: a cross-sectional study. J Clin Nurs. 2015;24(3–4):546–55.
42. Wo JY, Viswanathan AN. The impact of radiotherapy on fertility, pregnancy, and neonatal outcomes of female cancer patients. Int J Radiat Oncol Biol Phys. 2009;73(5):1304–12.
43. Gubbala K, Laios A, Gallos I, et al. Outcomes of ovarian transposition in gynaecological cancers; a systematic review and meta-analysis. J Ovarian Res. 2014;7(1):69.
44. Siahpush M, Singh GK. Sociodemographic predictors of pap test receipt, currency and knowledge among Australian women. Prev Med. 2002;35(4):362–8.
45. Greenwald HP, McCorkle R, Baumgartner K, et al. Quality of life and disparities among long-term cervical cancer survivors. J Cancer Surviv. 2014;8(3):419–26.
46. McGarvey EL, Baum LD, Pinkerton RC, et al. Psychological sequelae and alopecia among women with cancer. Cancer Pract. 2001;9(6):283–9.

47. Hesketh PJ, Batchelor D, Golant M, et al. Chemotherapy-induced alopecia: psychosocial impact and therapeutic approaches. Support Care Cancer. 2004;12(8):543–9.
48. Tageja N, Groninger H. Chemotherapy-induced nausea and vomiting: an overview and comparison of three consensus guidelines. Postgrad Med J. 2016;92(1083):34–40.
49. Lohr L. Chemotherapy-induced nausea and vomiting. Cancer J. 2008;14(2):85–93.
50. Jean-Pierre P, McDonald BC. Neuroepidemiology of cancer and treatment-related neurocognitive dysfunction in adult-onset cancer patients and survivors. Handb Clin Neurol. 2016;138:297–309.
51. Tewari KS, Sill MW, Penson RT, et al. Bevacizumab for advanced cervical cancer: final overall survival and adverse event analysis of a randomised, controlled, open-label, phase 3 trial (Gynecologic Oncology Group 240). Lancet. 2017;390(10103):1654–63.
52. Penson RT, Huang HQ, Wenzel LB, et al. Bevacizumab for advanced cervical cancer: patient-reported outcomes of a randomised, phase 3 trial (NRG Oncology-Gynecologic Oncology Group protocol 240). Lancet Oncol. 2015;16(3):301–11.
53. Datta NR, Samiei M, Bodis S. Radiation therapy infrastructure and human resources in low- and middle-income countries: present status and projections for 2020. Int J Radiat Oncol Biol Phys. 2014;89(3):448–57.
54. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69–90.
55. De Vuyst H, Alemany L, Lacey C, et al. The burden of human papillomavirus infections and related diseases in sub-Saharan Africa. Vaccine. 2013;31(5):F32–46.
56. Keller M, Sommerfeldt S, Fischer C, et al. Recognition of distress and psychiatric morbidity in cancer patients: a multi-method approach. Ann Oncol. 2004;15(8):1243–9.
57. Simard S, Thewes B, Humphris G, et al. Fear of cancer recurrence in adult cancer survivors: a systematic review of quantitative studies. J Cancer Surviv. 2013;7(3):300–22.
58. Conic I, Miljkovic S, Tosic-Golubovic S, et al. Anxiety levels related to the type of therapy for cervical cancer. Cent Eur J Med. 2012;7(4):490–6.
59. Hazewinkel MH, Sprangers MAG, Van Der Velden J, et al. Severe pelvic floor symptoms after cervical cancer treatment are predominantly associated with mental and physical well-being and body image: a cross-sectional study. Int J Gynecol Cancer. 2012;22(1):154–60.
60. Carter J, Rowland K, Chi D, et al. Gynecologic cancer treatment and the impact of cancer-related infertility. Gynecol Oncol. 2005;97(1):90–5.
61. Angarita AM, Johnson CA, Fader AN, et al. Fertility preservation: a key survivorship issue for young women with cancer. Front Oncol. 2016;6:102.
62. Chan JL, Letourneau J, Salem W, et al. Regret around fertility choices is decreased with pre-treatment counseling in gynecologic cancer patients. J Cancer Surviv. 2017;11(1):58–63.
63. Bakker RM, Kenter GG, Creutzberg CL, et al. Sexual distress and associated factors among cervical cancer survivors: a cross-sectional multicenter observational study. Psycho-Oncology. 2016;26(10):1470–7.
64. Lonnée-Hoffmann R, Pinas I. Effects of hysterectomy on sexual function. Curr Sexual Health Rep. 2014;6(4):244–51.
65. Vermeer WM, Bakker RM, Kenter GG, et al. Cervical cancer survivors' and partners' experiences with sexual dysfunction and psychosexual support. Support Care Cancer. 2016;24:1679–87.
66. Ghafoori F, Noughabi ZS, Sarafraz N, et al. Sexual outcomes in women with cervical cancer: a review article. Iran J Obstet Gynecol Infert. 2016;19(28):22–7.
67. Solbrække KN, Bondevik H. Absent organs—present selves: exploring embodiment and gender identity in young Norwegian women's accounts of hysterectomy. Int J Qual Stud Health, 2015;10. https://doi.org/10.3402/qhw.v10.26720.
68. Syse A, Kravdal Ø. Does cancer affect the divorce rate? Demogr Res. 2007;16(15):469–92.
69. De Groot JM, Mah K, Fyles A, et al. The psychosocial impact of cervical cancer among affected women and their partners. Int J Gynecol Cancer. 2005;15(5):918–25.
70. Maguire R, Kotronoulas G, Simpson M, et al. A systematic review of the supportive care needs of women living with and beyond cervical cancer. Gynecol Oncol. 2015;136(3):478–90.

71. Ye S, Yang J, Cao D, et al. A systematic review of quality of life and sexual function of patients with cervical cancer after treatment. Int J Gynecol Cancer. 2014;24(7):1146–57.
72. Alcala HE, Mitchell E, Keim-Malpass J. Adverse childhood experiences and cervical Cancer screening. J Womens Health (Larchmt). 2017;26(1):58–63.
73. Jayasinghe YL, Sasongko V, Lim RW, et al. The association between unwanted sexual experiences and early-onset cervical cancer and precancer by age 25: a case-control study. J Womens Health (Larchmt), 2016, 17. https://doi.org/10.1089/jwh.2016.5742.
74. Szender JB, Cannioto R, Gulati NR, et al. Impact of physical inactivity on risk of developing cancer of the uterine cervix: a case-control study. J Low Genit Tract Dis. 2016;20(3):230–3.
75. Gerritsen JK, Vincent AJ. Exercise improves quality of life in patients with cancer: a systematic review and meta-analysis of randomised controlled trials. Br J Sports Med. 2016;50(13):796–803.
76. Sawyer A. Complementary exercise and quality of life in patients with breast cancer. Br J Nurs. 2014;23(16):S18–23.
77. Aaronson NK, Ahmedzai S, Bergman B, et al. The European Organisation for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. J Natl Cancer Inst. 1993;85(5):365–76.
78. Flechtner H, Bottomley A. Quality of life assessment and research in the EORTC (European Organisation for Research and Treatment of Cancer). Oncologie. 2006;8(5):443–6.
79. Cherny NI, Sullivan R, Dafni U, et al. A standardised, generic, validated approach to stratify the magnitude of clinical benefit that can be anticipated from anti-cancer therapies: the European Society for Medical Oncology Magnitude of Clinical Benefit Scale (ESMO-MCBS). Ann Oncol. 2015;26(8):1547–73.
80. Schnipper LE, Davidson NE, Wollins DS, et al. American Society of Clinical Oncology statement: a conceptual framework to assess the value of cancer treatment options. J Clin Oncol. 2015;33(23):2563–77.
81. Gilbert A, Sebag-Montefiore D, Davidson S, et al. Use of patient-reported outcomes to measure symptoms and health related quality of life in the clinic. Gynecol Oncol. 2015;136(3):429–39.
82. Snyder CF, Aaronson NK, Choucair AK, et al. Implementing patient-reported outcomes assessment in clinical practice: a review of the options and considerations. Qual Life Res. 2012;21(8):1305–14.
83. Valderas JM, Kotzeva A, Espallargues M, et al. The impact of measuring patient-reported outcomes in clinical practice: a systematic review of the literature. Qual Life Res. 2008;17(2):179–93.
84. PROQOLID Patient-Reported Outcome and Quality of Life Instruments Database [Internet]. Mapi Research Trust. 2016 [cited 2016 November 28]. http://www.proqolid.org/instruments
85. King MT. A point of minimal important difference (MID): a critique of terminology and methods. Expert Rev Pharmacoecon Outcomes Res. 2011;11(2):171–84.
86. Streiner DL, Norman GR. Health measurement scales: a practical guide to their development and use. Oxford: Oxford University Press; 1996.
87. Cella D, Tulsky DS, Gray R, et al. The Functional Assessment of Cancer Therapy (FACT) scale: development and validation of the general measure. J Clin Oncol. 1993;11(3):570–9.
88. Greimel ER, Kuljanic Vlasic K, Waldenstrom AC, et al. The European Organization for Research and Treatment of Cancer (EORTC) quality-of-life questionnaire cervical cancer module: EORTC QLQ-CX24. Cancer. 2006;107(8):1812–22.
89. Ding Y, Hu Y, Hallberg IR. Psychometric properties of the Chinese version of the Functional Assessment of Cancer Therapy-Cervix (FACT-Cx) measuring health-related quality of life. Health Qual Life Outcomes. 2012;10(1):124.
90. Calhoun EA, Welshman EE, Chang CH, et al. Psychometric evaluation of the Functional Assessment of Cancer Therapy/Gynecologic Oncology Group-Neurotoxicity (Fact/GOG-Ntx) questionnaire for patients receiving systemic chemotherapy. Int J Gynecol Cancer. 2003;13(6):741–8.

91. Cella D, Peterman A, Hudgens S, et al. Measuring the side effects of taxane therapy in oncology: the functional assessment of cancer therapy-taxane (FACT-taxane). Cancer. 2003;98(4):822–31.
92. Levine M, Guyatt G, Gent M, et al. Quality of life in stage II breast cancer: an instrument for clinical trials. J Clin Oncol. 1988;6(12):1798–810.
93. Coates A, Glasziou P, McNeil D. On the receiving end – III. Measurement of quality of life during cancer chemotherapy. Ann Oncol. 1990;1:213–7.
94. Luckett T, King MT. Choosing patient-reported outcome measures for cancer clinical research – practical principles and an algorithm to assist non-specialist researchers. Eur J Cancer. 2010;46(18):3149–57.
95. Snyder CF, Watson ME, Jackson JD, et al. Patient-reported outcome instrument selection: designing a measurement strategy. Value Health. 2007;10:S76–85.
96. Luckett T, King MT, Butow PN, et al. Choosing between the EORTC QLQ-C30 and FACT-G for measuring health-related quality of life in cancer clinical research: issues, evidence and recommendations. Ann Oncol. 2011;22(10):2179–90.
97. Mercieca-Bebber R, Palmer MJ, Brundage M, et al. Design, implementation and reporting strategies to reduce the instance and impact of missing patient-reported outcome (PRO) data: a systematic review. BMJ Open. 2016;6(6):e010938.
98. Aaronson NK, Elliott TE, Greenhalgh J, et al. User's guide to implementing patient-reported outcomes assessment in clinical practice, Version 2: January: International Society for Quality of Life Research; 2015.

Index

S. A. Farghaly (ed.), *Uterine Cervical Cancer*,
https://doi.org/10.1007/978-3-030-02701-8