Cervical cancer heterogeneity: a constant battle against viruses and drugs

Qian Sun1†, Liangliang Wang1†, Cong Zhang1, Zhenya Hong2* and Zhiqiang Han1*

Abstract
Cervical cancer is the first identified human papillomavirus (HPV) associated cancer and the most promising malignancy to be eliminated. However, the ever-changing virus subtypes and acquired multiple drug resistance continue to induce failure of tumor prevention and treatment. The exploration of cervical cancer heterogeneity is the crucial way to achieve effective prevention and precise treatment. Tumor heterogeneity exists in various aspects including the immune clearance of viruses, tumorigenesis, neoplasm recurrence, metastasis and drug resistance. Tumor development and drug resistance are often driven by potential gene amplification and deletion, not only somatic genomic alterations, but also copy number amplifications, histone modification and DNA methylation. Genomic rearrangements may occur by selection effects from chemotherapy or radiotherapy which exhibits genetic intra-tumor heterogeneity in advanced cervical cancers. The combined application of cervical cancer therapeutic vaccine and immune checkpoint inhibitors has become an effective strategy to address the heterogeneity of treatment. In this review, we will integrate classic and recently updated epidemiological data on vaccination rates, screening rates, incidence and mortality of cervical cancer patients worldwide aiming to understand the current situation of disease prevention and control and identify the direction of urgent efforts. Additionally, we will focus on the tumor environment to summarize the conditions of immune clearance and gene integration after different HPV infections and to explore the genomic factors of tumor heterogeneity. Finally, we will make a thorough inquiry into completed and ongoing phase III clinical trials in cervical cancer and summarize molecular mechanisms of drug resistance among chemotherapy, radiotherapy, biotherapy, and immunotherapy.

Keywords: Human papillomavirus, Tumor heterogeneity, Tumor microenvironment, Drug resistance, Immunotherapy

Introduction
Human papillomavirus (HPV) causes an overwhelming majority of cervical cancers (CCs) and an alarmingly increased proportion of oropharyngeal cancers (OPCs). As the earliest discovered HPV-associated cancer, the tumorigenesis and infiltration of cervical cancer are closely relevant to the persistent infection and genome integration of HPV [1, 2]. Although with clear etiology, tumor heterogeneity still exists and gradually becomes a new challenge in the field of HPV-associated cancer research. Three concepts of heterogeneity need to be clarified: inter-patient heterogeneity, inter-tumoral heterogeneity, and intra-tumoral heterogeneity. Differences in tumor phenotypes and genotypes among individuals or distinct tumor sites are defined as inter-patient heterogeneity and inter-tumoral heterogeneity, and intra-tumoral heterogeneity. Differences in tumor phenotypes and genotypes among individuals or distinct tumor sites are defined as inter-patient heterogeneity and inter-tumoral heterogeneity respectively. In contrast, intra-tumoral heterogeneity means genomic, transcriptomic, epigenetic, or phenotypic differences within the same tumor lesion which are associated with therapeutic resistance...
and considerably more challenging [3]. Mendelian law of inheritance suggests that the free combination of genes is an important reason for the emergence of biodiversity [4]. As these probabilistic events encounter Darwinian adaptational selection over time, tumor cells and normal cells will continue to compete in different quadrants of time and space [5]. Genetic intra-tumoral heterogeneity, inter-tumoral heterogeneity, and inter-patient heterogeneity are reflected in a dynamic process of tumorigenesis, invasion, metastasis, or drug resistance [6–8]. To overcome the dilemma of precision therapy, it is necessary to break through each of these aspects. The rapid development of sequencing technology provides a platform for revealing tumor heterogeneity. Scanty knowledge has been uncovered on how heterogeneity plays roles in tumor pathogenesis and precision therapy until application of single-cell transcriptome analysis. The advances in single-cell RNA sequencing (scRNA-seq) include distinguishing neoplastic from normal tissue in individual patients and different disease states [9, 10].

In this review, we explore the heterogeneity of cervical cancers from the perspectives of HPV-induced tumorigenesis, internal changes of human genome and molecular mechanisms of drug resistance. The molecular and clinical features of cervical squamous cell carcinoma are discussed in major. In addition, cancer stem cells, cervical adenocarcinoma, and neuroendocrine carcinoma are described respectively in the last chapter. Firstly, we emphasize the significant contribution of alterations of genetic material and HPV gene integration differences in tumorigenesis. Furthermore, we summarize the mechanisms of intra-tumoral and inter-tumoral heterogeneity among inchoate and advanced cancers. Finally, we attempt to explain the huge differences in resistance to therapies among populations through tumor heterogeneity and provide feasible strategies for precise treatment.

**Inferring heterogeneity with HPV**

**Heterogeneity of geographical distribution**

In the 1980s, German pathologist Dr Hausen identified HPV as the explicit cause of cervical cancer which opened a new revolution in the etiology treatment of cancer. Moreover, HPV infection is also the cause of multiple cancers in both women and men, including anogenital cancer (anal, vaginal, vulvar, and penial) or head and neck cancer (oropharynx, oral and laryngeal) [11]. Recent studies indicate that over 90% of cervical and anal cancers, over 70% of oropharynx cancers, about 70% of vulvar and vaginal cancers, together with more than 60% of penile cancers are related to HPV [12, 13].

Cervical cancer is the fourth most common cancer in women with 604,127 new cases and 341,831 deaths occurring worldwide in 2020 [14, 15]. The incidence and mortality have shown an obvious geographical imbalance between low-income and middle-income countries (LMICs) with high-income countries in cervical cancer patients. In LMICs, CC is the second most common cancer with an incidence rate of 18.8 per 100 000 women and a mortality rate of 12.4 per 100 000 women. In contrast, as a result of the availability of HPV prophylactic vaccines and standardized screening strategies, the incidence (11.3/100 000 women) and mortality (5.2/100000 women) of cervical cancer have decreased in high-income countries [14]. Vaccination and screening are effective in preventing cervical cancer, but they will impose a huge global economic burden. A systematic review has demonstrated that 106 million women have received at least one dose of HPV vaccine worldwide till 2014, but the HPV vaccination and standardized screening coverage in LMICs are still obviously low [16, 17]. The world health organization (WHO) made a call for global action toward CC elimination in 2018, through vaccinating 90% of all girls under the age of fifteen, screening 70% of women at the age of 25, and treating 90% of precancerous lesions. The prediction simulation using the WHO Cervical Cancer Elimination Modelling Consortium (CCEMC) shows that the premature mortality rate of CC in 78 LMICs could be reduced by a third in the next 10 years. The WHO triple-intervention strategy would result in a 96.2% reduction by 2070, and 98.6% reduction by 2120. Famously, vaccination alone could reduce the mortality by 62.7% till 2070 and 89.5% till 2120. It is believed that with concerted global efforts, the incidence of cervical cancer in LMICs will be steadily reduced (Fig. 1) [18]. In 2019, the first domestic bivalent HPV vaccine was released and contributed to the HPV vaccination program in China [19]. This geographical distribution heterogeneity is therefore bound to become uniform gradually with the improvement of the global economic level and the implementation of prevention strategies.

**Heterogeneity of HPV infection types**

The infection rate besides the infection site of different HPV types is heterogeneous across populations. Fifteen high-risk HPV (HR-HPV) types have been confirmed as carcinogenic viruses, as follows, 16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82. Among them, the cumulative infection rate of HPV16 and 18 accounts for 79% of the squamous-cell carcinomas, and accounts for 95% of the squamous-cell carcinomas together with HPV45, 31, 33, 52, 58, and 35 [20, 21]. A meta-analysis collated data from 115,789 HPV-positive patients has been performed to analyze the distribution of HR-HPV. The percentage of 13 HR-HPV infection distributions under different disease states are demonstrated in Fig. 2, and include HPV16, 18, 31,33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.
Among them, HPV16, 18 and 45 infections dominate in invasive cervical cancer (ICC) (ICC: normal ratios 3.1, 1.9 and 1.1, respectively) [22].

Another systematic meta-analysis collated data from 19,883 HIV-positive patients has been performed and analyzed the distribution of HR-HPV in 2017. Similarly, the ICC: normal ratios of HPV infections are 3.7 (HPV16), 2.5 (HPV18), and 2.6 (HPV45) respectively which is consistent with the conclusion in HIV-uninfected populations [23]. It is illustrated that HPV16, 18 and 45 positivity increase distinctly from normal cytology through squamous intraepithelial lesions to invasive cervical cancer which suggests that we should pay special attention to these types in cervical cancer screening.

On the other hand, in a large sample of healthy people screening, there is data to support a shift in the pre- and post-vaccine prevalence profile. HPV16, 18, 31, 52 and 58 were the most five common infection types in women with normal cytology in the pre-vaccine era [24]. However, infection rates of HPV52, 58, and 56 are increasing in the post-vaccine era [25, 26]. Whether bivalent and quadrivalent vaccines can provide cross-protection is controversial, but there is no doubt that the spectrum of HPV-associated squamous intraepithelial lesions and invasive cervical cancer will continue changing with the introduction of the 9-valent vaccine or even the 11-valent vaccine. Although cervical cancer is being treated earlier and earlier, it is still a constant battle against the ever-changing virus types.

Heterogeneity of anti-viral immunity
Upon HPV infection, the host cell immediately activates the innate and adaptive immunity to eliminate the virus [27]. Tumor heterogeneity of cervical cancer is reflected in the outcome of the battle between our immune system and virus invasion in the post infection
microenvironment (PIM). HPV is undoubtedly one of the most important external factors mediating the heterogeneity of tumor development. HR-HPV type, duration of infection, virulence, human genomic instability and immune clearance will affect the tumorigenesis and development of carcinoma [28]. There are three outcomes of the battle between our immune system and HPV infection. Firstly, the virus is thoroughly cleared by our immune system. Secondly, the overwhelming majority of the virus is cleared, and only minority viruses that lie dormant can escape immunological recognition. Thirdly, the virus escapes immune recognition and integrates into the human genome, resulting in persistent infections and tumorigenesis [29, 30]. Fortunately, persistent high-risk HPV (HR-HPV) infection combined with oncogene genomic integration might lead development of normal cervical cells into intraepithelial neoplasia (CIN) or ICC in decades (Fig. 3).

PIM has been recognized as a complex and dynamic position with a collection of highly heterogenous cellular or molecular compounds, especially induced by the interaction between HPV-infected keratinocytes and immune cells. Specific cellular immune reactions and break down of immunosuppressive status are essential for effective virus clearance. Insufficient trafficking or maturation of Langerhans cells may lead to antigen-presenting disorder
and CD8+ cytotoxic T lymphocyte (CTL) response impairment [31, 32]. Otherwise, the expression of MHC-I on the surface of keratinocytes have been down-regulated after HPV infection and recognition of CTLs will be avoided [33]. Except for CTLs, CD4+ T cells are essential in HPV clearance. An imbalance in T-helper 1(Th1) and Th2-type CD4+ T cells might be associated with immune dysregulation. Furthermore, the malfunction of NK cells is associated with immunosuppression [34].

**Heterogeneity of genomic instability and HPV integration**

Key characteristics of PIM include immunosuppressive state, oxidative stress response, extracellular matrix (ECM) remodeling, and metabolic reprogramming [35]. Oxidative stress could amplify inflammatory responses and result in accumulation of DNA damage, mutations or genome instability [36]. Expression of matrix metalloproteases is also increased and associated with ECM remodeling and precancerous lesion occurrence [37]. Once the immune microenvironment remodeling that promotes the persistence of HPV infection is established, genomic integration and cytopathic changes occur continuously.

As the overexpression of oncoproteins E6 and E7 in the HPV-infected keratinocytes, E6 disrupts p53 degradation and alteration of cell regulation, on the other hand, E7 induces retinoblastoma (pRb) degradation and promotes cell proliferation [38]. E6 and E7 may also induce genomic instability and lead to carcinogenesis by abrogating cell-cycle checkpoints [39]. Growing evidence suggests that chromosomal instability is also a driving force for the oncogenic transformation of cervical cancer. High chromosomal instability Hela cells exhibit a higher karyotype heterogeneity and are related to KRAS signaling regulation [40].

HPV is a small double-stranded DNA virus whose DNA fragments have the ability to integrate into the human genome. Associations between HPV integration and adjacent host genomic structural variation have been confirmed in HPV-positive cervical cancer cell lines. HPV16 integration has been detected firstly on chromosome 13 q22 in SiHa cell lines in 1987 [41]. HPV 16, 18, and 33 viral integration has been detected in cervical squamous cell carcinomas by scientists as early as 1991 [42]. All integration events of the 13 HR-HPV subtypes have already been observed, and an unbalanced distribution of HR-HPV genotypes in cervical cancer has been detected. We have summarized six high-quality studies with HPV integration data of cervical cancer patients through next-generation sequencing (NGS) or whole-genome sequencing (WGS), and the proportion of integration events among different subtypes is analyzed. We can see that the integration of type 16 and 18 accounts for more than 80% of all samples. Other HR-HPVs are HPV45, 31, 33, 52, 58, 59, 39, 56, 68, 35 and 51 in a descending order of integration ratio (Fig. 4) [43–48]. We have summarized the high-frequency (more than 4 reported) disrupted genes by HPV integration and listed the hotspots, such as 3 q28, 8 q24, and 13 q22. The top five reported genes are MACROD2, FHIT, POU5F1B, LRP1B and RAD51B (Table 1).

HPV integration normally breaks up the open reading frames of viral E1 and E2 genes which leads to the upregulation of E6 and E7 oncogenes [49]. Genomic instability, HPV integration and gain of telomerase at chromosome 3q26 appear to be strongly associated with genetic events in malignant transformation from CIN to invasive cervical carcinoma. In particular, chromosomal instability may precede genomic integration of oncogenic HPV, while increasing the human telomerase gene copy number occurs after integration as a termination product [50–52]. The integration hotspots are non-random and numerous microRNAs are located in the vicinity of integration hotspots and are influenced by the integrated HPV DNA. Highly homologous stretches of HPV16 viral gene E5 and L2 have been detected at the integration hotspots in independent patients which support themselves as quite important events in the integration process [53]. HPV E6E7 alternative transcripts have shown frequent isoforms in HPV16 or HPV18 positive cervical cancer [54]. Multiple frequent integration sites in human genome have been reported and verified through whole genome sequencing, high-throughput RNA, or chromosome conformation capture (Hi-C) sequencing, whereas the patterns of HPV integration in DNA and RNA samples differ significantly. For
Table 1 Summary of high-frequency disrupted genes by HPV integration in cervical cancer

| Gene   | Integrations reported | Official full name                                      | Gene ID | Location | Reference |
|--------|-----------------------|--------------------------------------------------------|---------|----------|-----------|
| MACROD2 | 13                    | Mono-ADP ribosylhydrolase 2                           | 140733  | 20p12.1  | [39–44]   |
| FHIT    | 11                    | Fragile histidine triad diadenosine triphosphatase     | 2272    | 3p14.2   | [39, 41, 42] |
| POU5F1B | 11                    | POU class 5 homeobox 1B                               | 5462    | 8q24.21  | [41–43]   |
| LRP1B   | 10                    | LDL receptor related protein 1B                       | 53353   | 2q22.1   | [39, 42]   |
| RAD51B  | 10                    | RAD51 paralog B                                       | 5890    | 14q24.1  | [39, 40, 44] |
| KLF12   | 9                     | Kruppel like factor 12                                | 11278   | 13q22.1  | [41, 42]   |
| KLF5    | 9                     | Kruppel like factor 5                                 | 688     | 13q22.1  | [39, 41, 42] |
| HMGA2   | 7                     | High mobility group AT-hook 2                         | 8091    | 12q14.3  | [42]       |
| ERBB2   | 7                     | Erb-b2 receptor tyrosine kinase 2                     | 2064    | 17q21.31 | [40, 41, 44] |
| DMD     | 7                     | Dystrophin                                            | 1756    | Xp21.2-p21.1 | [39, 42, 44] |
| MAPK10  | 6                     | Mitogen-activated protein kinase 10                    | 5602    | 4q21.3   | [39, 42, 43] |
| MYC     | 6                     | MYC proto-oncogene, bHLH transcription factor          | 4609    | 8q24.21  | [39, 40, 42, 44] |
| DLG2    | 6                     | Discs large MAGUK scaffold protein 2                  | 1740    | 11q14.1  | [39, 42]   |
| LEPRE1  | 6                     | Prolyl 3-hydroxyylase 2                                | 55214   | 3q28     | [39]       |
| CASC8*  | 9                     | Cancer susceptibility 8                               | 727677  | 8q24.21  | [39, 40, 44] |
| TP63    | 6                     | Tumor protein p63                                      | 8626    | 3q28     | [39, 40, 42, 44] |
| ENTPDS  | 5                     | Ectonucleoside triphosphate diphosphohydrolase 5      | 957     | 14q24.3  | [39]       |
| PAR3B   | 5                     | Par-3 family cell polarity regulator beta              | 117583  | 2q33.3   | [41, 42]   |
| PVT1*   | 5                     | PVT1 oncogene                                         | 5820    | 8q24.21  | [44]       |
| SEMA3D  | 5                     | Semaphorin 3D                                         | 223117  | 7q21.11  | [42]       |
| ZFAND3  | 5                     | Zinc finger AN1-type containing 3                     | 60865   | 6p21.2   | [39, 42]   |
| FOXP2   | 5                     | Forkhead box P2                                       | 93986   | 7q31.1   | [42, 44]   |
| PAKN    | 5                     | Parkin RBR E3 ubiquitin protein ligase                 | 5071    | 6q26     | [42, 44]   |
| TAF5    | 5                     | TAF4 chemokine like family member 5                   | 25817   | 22q13.32 | [39, 42, 44] |
| TPRG1   | 5                     | Tumor protein p63 regulated 1                         | 285386  | 3q28     | [39, 42, 43] |
| ARAP2   | 4                     | ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2 | 116984  | 4p14     | [39, 42]   |
| BB59    | 4                     | Bardet-Biedl syndrome 9                               | 27242   | 7p14.3   | [39, 42]   |
| CHL1    | 4                     | Cell adhesion molecule L1 like                         | 10752   | 3p26.3   | [39, 42]   |
| CNTNAP2 | 4                     | Contactin associated protein 2                        | 26047   | 7q35-q36.1 | [39, 42] |
| AGTR2,  | 4                     | Angiotensin II receptor type 2                         | 186     | Xq23     | [42]       |
| CADM2   | 4                     | Cell adhesion molecule 2                              | 253559  | 3p12.1   | [42]       |
| CDH7    | 4                     | Cadherin 7                                            | 1005    | 18q22.1  | [42]       |
| CPNE8   | 4                     | Copine 8                                              | 144402  | 12q12    | [42]       |
| DCC     | 4                     | DCC nemtin 1 receptor                                  | 1630    | 18q21.2  | [42]       |
| DUSP6   | 4                     | Dual specificity phosphatase 6                        | 1848    | 12q23.1  | [42]       |
| EPAH6   | 4                     | EPH receptor A6                                       | 285220  | 3q11.2   | [42]       |
| HS5ST4  | 4                     | Heparan sulfate-glucosamine 3-sulfotransferase 4      | 9951    | 16p12.1  | [42]       |
| TEKT4P2*| 4                     | Tektin 4 pseudogene 2                                 | 100132288 | 21p11.2 | [42]       |
| MSX2    | 4                     | Msh homeobox 2                                        | 4488    | 5q35.2   | [42]       |
| NEK11   | 4                     | NIMA related kinase 11                                | 79859   | 3q22.1   | [42]       |
| PCDH15  | 4                     | Protocadherin related 15                              | 65217   | 10q21.1  | [42]       |
| PL35    | 4                     | Plastin 3                                             | 5358    | Xq23     | [42]       |
| PRDM9   | 4                     | PR/SET domain 9                                       | 56979   | 5p14.2   | [42]       |
| ZNF33B  | 4                     | Zinc finger protein 33B                               | 7582    | 10q11.21 | [42]       |
| IGF1    | 4                     | Insulin like growth factor 1                          | 3479    | 12q23.2  | [42, 44]   |
| CNTNAP5 | 4                     | Contactin associated protein family member 5          | 120684  | 2q14.3   | [39, 42]   |
| ERC2    | 4                     | ELKS/RAB6-interacting/CAST family member 2           | 26059   | 3p14.3   | [39, 42]   |
| FGFI13  | 4                     | Fibroblast growth factor 13                           | 2258    | Xq26.3-q27.1 | [39, 42] |
| LINGO2  | 4                     | Leucine rich repeat and Ig domain containing 2        | 158038  | 9p21.2-p21.1 | [39, 42] |
instance, DLG2, FHIT, HMGA2, KLF12, KLF5, LRP1B, LEPREL1, LINC00392, POU5F1B, and SEMA3D are DNA hotspots [41, 46]. In addition, CASC8, CASC21, ERBB2, RAD51B, RAP2B, TEX41, TP63, TTC6, MACROD2, MIPOL1, and MYC are hotspot genes in RNA samples [55]. DNA breakpoints are prone to an intron, in contrast, RNA breakpoints are prone to the region of EXON [56]. CCDC106 integration on chromosome 19 has been exhibited in altering local chromosome architecture and structure remodeling [57]. Additionally, the changes in protein expression levels after HPV integration are inconsistent. FHIT and LRP1B are downregulated, while MYC and HMGA2 are elevated. Moreover, the fusion between HPV and human genome may have occurred by microhomology-mediated DNA repair pathways [46].

In terms of prognostic analysis, HPV16 positive status of the pelvic lymph nodes is a significant predictor of recurrent cervical cancer, while HPV16 integrated form is an unfavorable predictor of overall survival [58, 59]. HPV-DNA integration has been detected with association in carcinogenesis and recurrence free survival [60]. HPV integration into the common fragile sites may be associated with distant metastasis [61]. Accurate detection of integration sites will continue with the improvement and combination of multidimensional technologies, such as nanopore sequencing and fluorescent in situ hybridization [62, 63]. Detection methods for viral integration sites are changing rapidly, and we believe that the blueprint for HPV integration will become clearer in the next decades.

**Inferring heterogeneity with human genomics**

Genetic intra-tumor heterogeneity acts as a key challenge in tumor evolution and management which affects patients’ outcomes [6, 64, 65]. The fundamental biological mechanisms underlying intra-tumor heterogeneity include genetic drift, selection, heritable variation, and environmental changes [66, 67]. Somatic mutation of FGFR3 has been identified in a large proportion of cervical cancer by Cappellen et al. as early as 1999 [68]. Nevertheless, at least three driver gene alterations are necessary to convert normal cells to malignant cells [69]. Over the past decades, multiple gene expression profiles and novel through-out sequencing studies have focused on capturing intra-tumor heterogeneity over time and space [70, 71]. Whole genome sequencing data among pan-cancer patients (including cervical cancer) has identified 95.1% subclonal expansions of 1705 tumors which verified the importance of intra-tumor heterogeneity [72]. Several scRNA-seq analyses have also been performed to study intra-tumor heterogeneity at the level of individual cells in cervical cancer. We summarize the intra-tumor heterogeneity of cervical cancer from genomic, transcriptomic and epigenetic alterations under different approaches.

### Table 1 (continued)

| Gene     | Integrations reported | Official full name                                      | Gene ID | Location          | Reference |
|----------|-----------------------|--------------------------------------------------------|---------|-------------------|-----------|
| RPRD2    | 4                     | Regulation of nuclear pre-mRNA domain containing 2     | 23248   | 1q21.2            | [39]      |
| MYO16    | 4                     | Myosin XVI                                             | 23026   | 13q33.3           | [39, 42]  |
| PTPRN2   | 4                     | Protein tyrosine phosphatase receptor type N2          | 5799    | 7q36.3            | [39, 42]  |
| RELN     | 4                     | Reelin                                                 | 5649    | 7q22.1            | [39, 42]  |
| RG56     | 4                     | Regulator of G protein signaling 6                     | 9628    | 14q24.2           | [39, 42]  |
| SPOCK3   | 4                     | SPARC (osteonectin), cvcc and kazal like domains proteoglycan 3 | 50859   | 4q32.3            | [39, 42]  |
| ZFAT     | 4                     | Zinc finger and AT-hook domain containing              | 57623   | 8q24.22           | [39, 42]  |
| CSMD3    | 4                     | CUB and Sushi multiple domains 3                       | 114788  | 8q23.3            | [39, 41, 42] |
| ERBB4    | 4                     | Erb-b2 receptor tyrosine kinase 4                      | 2066    | 2q34              | [39, 41, 42] |
| CA10     | 4                     | Carbonic anhydrase 10                                  | 56934   | 17q21.33-q22      | [39, 42]  |
| PDE4D    | 4                     | Phosphodiesterase 4D                                   | 5144    | 5q11.2-q11.21     | [39, 42]  |
| NLGN1    | 4                     | Neuroligin 1                                           | 22871   | 3q26.31           | [39, 42]  |
| PROX1    | 4                     | Prospero homeobox 1                                    | 5629    | 1q32.3            | [40, 42]  |
| ZMAT4    | 4                     | Zinc finger matrix-type 4                              | 79698   | 8p11.21           | [39, 42]  |
| TNIK     | 4                     | TRAF2 and NCK interacting kinase                       | 23043   | 3q26.2-q26.31     | [39, 40]  |

* Represent the gene type is ncRNA or pseudo, others are protein coding genes.
Somatic genomic alterations in cervical cancer
The Catalogue Of Somatic Mutations In Cancer (COSMIC) is the world’s most comprehensive repository of human cancer somatic mutations [73]. Driver hotspots from COSMIC single-base substitution (SBS) mutational signatures are classified into four categories: Deamination, APOBEC, somatic hypermutation, and signature SBS39 [74]. The apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) which converts cytosine to uracil during RNA editing and retrovirus restriction, has been confirmed in mediating pervasive mutagenesis in human cancers [75]. APOBEC-associated hotspots consist of one to two specific point mutations. In contrast, hotspots associated with somatic hypermutation are characterized by somatic single nucleotide variant (sSNV) clusters in promoter regions, which are clusters of variations in a single nucleotide without any limitations of frequency arisen in somatic cells. APOBEC mutagenesis pattern is associated with 34 common mutational hotspots across multiple cancers and has been identified as the predominant source of mutations in cervical cancers [74, 76]. The high-throughput genotyping platform has been used to interrogate cervical tumors and the consistently high mutation rates of PIK3CA have been confirmed. The APOBEC mutagenesis pattern is associated with nucleotide substitution in the E542K or E545K of PIK3CA, while the non-APOBEC mutagenesis pattern coexists at the same time [44].

The recognized mutated genes are ARID1A, CASP8, EP300, ERBB3, FBXW7, HLA-A, HLA-B, KRAS, MAPK1, NFE2L2, PIK3CA, PTEN, SHKBP1 and TGFBR2 in cervical cancer. It’s worth noting that over 70% of CCs exhibit genomic alterations in PI3K-MAPK and TGFβ signaling pathways [44, 77]. Novel significantly mutated genes have been discovered through deep RNA sequencing approaches and clustering of their mutant allele fraction variants. At least 20% of cervical cancers harbor somatic LKB1 mutations. Approximately 100% of tumors with these mutations harbored single nucleotide substitutions, identifiable monoallelic or biallelic deletions or multiplex ligation site amplification (MLPA) [78]. Mutational sequencing has identified that 40% of 23 cervical cancer specimens harbored somatic mutations of NOL7, a tumor suppressor gene located on 6p23. Multiple CpG dinucleotides have been detected spanning the first exon or the 5’ untranslated region of NOL7, resulting in its inactivation [79].

There is heterogeneity in gene mutations among different pathological types. PIK3CA mutation rates keep consistent between adenocarcinomas and squamous cell carcinomas. The major mutations in squamous cell carcinomas include EP300, FBXW7, MAPK1, NFE2L2 and EGFR, while KRAS, ELF3, and CBFB in adenocarcinoma [80, 81]. The Cancer Genome Atlas (TCGA) Research Network has identified high frequencies of ARID1A, KRAS, and PTEN mutations in endometrial-like cervical cancers [48]. Mutations in PIK3CA, KRAS, and TP53 have also been detected most commonly in small cell cervical cancer using next generation sequencing [82].

Differential gene expressions in cervical cancer
To discover transcriptomic intra-tumor heterogeneity, previous studies have investigated differential transcript gene expressions between normal and cervical cancer tissues through microarray technologies [83–88]. At the RNA level, gene expressions determined by the expression profiling microarray are detected by reverse transcription-polymerase chain reaction (RT-PCR). While at the protein level, the expressions of specific proteins are often described in immunohistochemical (IHC) staining. Multiple-gene transcript signature with differential expressions by cDNA microarray could be used for molecular classification between stage IB and IIB and prediction of response to radiotherapy for advanced cervical cancer [85, 86, 89]. Differential expressions of CDKN2A and PTGES have been identified in invasive cervical cancer versus normal keratinocytes through oligonucleotide microarrays and confirmed through immunohistochemical staining [90]. Apoptotic genes BCL2, BCL2L1, and BIRC2 have been identified as upregulated in late-stage cervical cancer compared to early-stage cases [91]. DPP4, EDN3, FGF14, TAC1 and WNT16 have been indicated simultaneously downregulated and hypermethylated in cervical cancer [92]. Message RNA expression levels of RhoB and STMM1 have been validated associated with overall survival in cervical cancer [93]. A positive correlation has been observed between gene expression of HPV E6/E7 oncogenes and UHMK1 [94].

Expression profiling has been replaced gradually by more accurate sequencing techniques and the search for differential expressed genes (DEGs) in tumors continues. Three DEGs, including RDH12, UBD, and SAA1 have been screened with correlation to tumor size, lymphatic metastasis, and depth of cervical invasion in cervical squamous cell carcinoma through RNA sequencing [95]. Upregulated expression of AKT3 in cervical cancer has been related to resistance to cisplatin [96]. Transcriptome sequencing in HPV16 positive cervical cancer tissues has identified 140 DEGs enriched in cell cycle and DNA repair [97].

Heterogeneity analyzed by single-cell RNA sequencing approaches
Single-cell sequencing is a promising systematic and comprehensive approach to delineating subclone
associations and intratumor heterogeneity. Conclusions of single-cell sequencing researches have provided a deeper understanding of specific mechanisms leading to heterogeneity in recent years. The landscape of heterogeneity within 22 cancer cell lines has identified twelve recurrent heterogeneous programs (RHPs) even without the native tumor microenvironment. These RHPs are associated with cell cycle, stress responses, epithelial-mesenchymal transition, and protein metabolism [98]. Focusing on cervical cancer, single-cell RNA sequencing data of 20,938 cells have divided tumor cells into four subpopulations with distinct signature genes and prognoses. Specifically, the cells in the first subpopulation are enriched in immune regulation signaling pathways, such as the ErbB signaling pathway; the cells in the third subpopulation are suggested with high proliferative activity because of their high expression of MKI67, CCNB1 and TOP2A genes. The last two subpopulations are regarded as the original cancer cells and the terminal cancer cells respectively, one with over-expressed stem-related genes SOX2 and ALDH1A1 and the other with high expressions of genes enriched in steroid biosynthesis, mismatch repair and peroxisome pathways [99]. Another single-cell RNA sequencing data of 24,371 cells aiming to comprehensively analyze chemotherapy resistant cervical cancer cells have clustered cells into nine subpopulations. Differentially expressed genes enriched in the PI3K/AKT pathway are involved in chemotherapy resistance [100]. The main limitations of microarray and sequencing technologies are detecting variations at the DNA or RNA level rather than the protein level. Validation studies in conjunction with proteomics are essential. Cellular heterogeneity is being characterized in cervical cancer with the advent of single-cell genomics which may provide more accurate information on cancer characteristics, prognostic prediction, and treatment decision selection.

**Epigenetic landscape in cervical cancer**

Tumor development and drug resistance are sometimes driven by potential gene amplification and deletion, not only somatic genomic alterations but also copy number amplifications, histone modification, and DNA methylation. A large-scale genomic study, including genomic, transcriptomic, and epigenomic landscapes of 118 Ugandan cervical cancer patients has been performed. DNA methylation, histone marks, and gene expression dysregulation differ between A9 and A7 HPV clades. Clade A7 corresponded to a less differentiated phenotype of cervical cancer and lead to a poorer prognosis. Changes in histone modification are associated with HPV integration [101]. Another comprehensive genomic analysis including whole exome sequencing, copy number and methylation analysis of 228 primary cervical cancers has revealed amplifications in immune checkpoint genes PD-L1 and PD-L2, together with lapatinib associated gene BCAR4 [102]. A C-score model according to the chromosomal-arm-level copy number alterations (CNAs) changes of 1q, 2q, 3p, and 7q has been validated to distinguish ICC from normal tissues with 100% sensitivity and specificity [103].

Deregulation of micro-RNA (miRNA), long non-coding RNA (lncRNA) and circular RNA (circRNA) have also been revealed in cervical cancer patients in recent researches. Specifically, miRNAs are small non-coding RNAs which can regulate gene expression through binding to DNA or mRNA [104]. While lncRNAs are long non-coding RNAs which can regulate gene transcription mediated by interacting with chromatin-modifying complexes and miRNAs [105]. CircRNAs are also small non-coding RNAs playing big parts in post-transcription and participate in genetic expression [106]. A type of endogenous RNA, specifically, competing endogenous RNAs (ceRNAs) have been identified to influent the target genes by miRNA and participate in cancer regulation process ultimately [107]. The ceRNA-miRNA-mRNA regulatory axis is gradually explored in cervical cancer research. Both lncRNAs and circRNAs may function as sponges or ceRNAs of miRNAs to regulate mRNA expression [108]. A recent review summarized the reciprocal regulation role of miRNAs, IncRNAs and circRNAs in CC patients. The miRNAs are divided into “oncogenic” miRNAs (miR-10a, miR-19, miR-21, and miR-146a et al.) and “tumor suppressive” miRNAs (miR-29a, miR-214, miR-218, and miR-372 et al.) [109]. Around 14 IncRNAs have shown to be altered and affected important metabolic pathways such as STAT3, wnt/β-catenin, PI3K/AKT, and Notch signaling in cervical cancer [110]. LncRNA XLOC_006390 can serve as a ceRNA and has been verified reversely regulating the expression of miR-331-3p and miR-338-3p, and facilitating tumorigenesis or metastasis in cervical cancer [111]. CircRNA_VPRBP regulates miR-93-5p/FRMD6 axis which lead to inhibited proliferation, migration and invasion of cervical cancer cells [112]. Furthermore, circRNA hsa_circ_0000515 acts as a miR-326 sponge, has been demonstrated to promote cervical cancer progression through upregulated ELK1 expression [113]. These findings might enumerate the regulatory mechanisms of epigenetics in the development of cervical cancer. However, the complexity interaction among diverse non-coding RNAs shows great heterogeneity, which still needs to be further verified.

**Inferring heterogeneity with therapeutic diversity**

HPV screening and classic three-step diagnostic criteria have been quite normalized and widely used worldwide in the detection of early-stage cervical cancer. According to clinical guidelines, standard surgical treatment is the
Heterogeneity in terms of chemotherapy resistance

Cervical cancer chemotherapy can be divided into neoadjuvant chemotherapy (NACT) aiming to shrink the mass to facilitate operation, adjuvant chemotherapy or concurrent chemoradiotherapy (CCRT) as maintenance after surgical treatment or standard treatment for locally advanced patients, and palliative chemotherapy for relieving symptoms, pain or prolonging survival in recurrent or metastatic patients [117]. The majority of these patients will receive more than two combination treatments. Most studies on drug resistance have been limited to in vitro experiments, and few studies have been validated in drug-resistant populations. The molecular mechanism of chemotherapeutic resistance has not been fully understood but could be speculated via blocking DNA damage repair, oxidative stress, autophagy, and apoptosis signaling pathways. Both coding and non-coding RNAs participate in chemoresistance. Non-coding RNAs, including miRNA, IncRNA, and circRNA, are potential therapeutic targets in cancer treatment development. However, its role in the field of drug resistance of cervical cancer remains to be further explored. Genomic rearrangements may occur by selecting effects from chemoradiotherapy which exhibits genetic intra-tumor heterogeneity in advanced cervical cancers. Platinum-paclitaxel combination chemotherapy is recommended as the first-line chemotherapy drugs in multiple solid cancers and we explain their mechanisms of chemotherapy resistance individually [118].

Cisplatin has been used in most studies of platinum resistance. The mechanisms underlying cisplatin resistance in CC are respectively DNA damage repair increase, apoptosis inactivation, epithelial-mesenchymal transition activation, and DNA methylation alteration [119]. For

Table 2 Completed randomized controlled Phase III trials in cervical cancer

| Trial identifier | Brief title | Actual Enrolment | Stage | Arm | Outcomes |
|-----------------|-------------|-----------------|-------|-----|----------|
| NCT00002536     | Surgery with or without chemotherapy in treating patients with stage IB cervical cancer | 288   | IB    | Arm I: RHPPL, Arm II: NACT + RHPPL | Not statistically significant |
| NCT00191100     | Comparative study of gemcitabine, cisplatin and radiation versus cisplatin and radiation in cancer of the cervix | 515   | IIB, IVA | Arm I: Gemcitabine + Cisplatin + Brachytherapy, Arm II: Cisplatin + Brachytherapy | PFS (HR = 0.68; 95% CI: 0.49–0.95; p = 0.0227) and OS (HR = 0.68; 95% CI: 0.49–0.95; p = 0.0224) were improved in arm I vs arm II |
| NCT00803062     | Paclitaxel and cisplatin or topotecan with or without bevacizumab in treating patients with stage IVC, recurrent, or persistent cervical cancer | 452   | IVC, recurrent, persistent | Arm I: Bevacizumab + Chemotherapy, Arm II: Chemotherapy | Median OS was improved in arm I vs arm II (17.0 vs. 13.3 months, HR = 0.71; 95% CI = 0.54–0.95, p = 0.004) |
| NCT0003945      | Comparison of three chemotherapy regimens in treating patients with stage IVB, recurrent, or persistent cervical cancer | 294   | IVB, recurrent, persistent | Arm I: Cisplatin + Topotecan, Arm II: Cisplatin | Median OS (94 vs. 6.5 months, P = 0.017) and PFS (46 vs. 2.9 months, P = 0.014) were improved in arm I vs arm II |
| NCT0004077      | Comparison of four combination chemotherapy regimens using cisplatin in treating patients with stage IVB, recurrent, or persistent cancer of the cervix | 513   | IVB, recurrent, persistent | Arm I: Paclitaxel + Cisplatin, Arm II: Vinorelbine + Cisplatin, Arm III: Gemcitabine + Cisplatin, Arm IV: Topotecan + Cisplatin | Best OS (12.87 months) and PFS (5.82 months) in arm I |

RHPPL: Radical hysterectomy and pelvic and para-aortic lymphadenectomy, NACT: Neoadjuvant chemotherapy, PFS: Progression free survival, HR: Hazard ratio, CI: Confidence interval, OS: Overall survival
instance, the upregulated expression of COX-2 has been assessed with neoadjuvant cisplatin-based resistance and unfavorable overall survival in locally advanced CC patients [120]. Cisplatin induces chemotherapy resistance of well-differentiated cell line Caski cells by upregulating Src family kinase and interaction with EphA4 through the reactive oxygen species pathway [121]. Inhibiting endogenous EZH2 expression has shown decreased cell metastasis, reversed cisplatin resistance in HeLa cells, and increased antitumor effects in nude mice. Interfering EZH2 expression has been identified correlated with Dicer overexpressed or regulated H3K27 methylation level, which exhibit antitumor activities by interfering the progression of miRNA transcription, and cell cycle and promote cell apoptosis [122]. MALAT1 and PSAT1 could induce resistance in SiHa cells through PI3K/Akt pathway [123, 124]. GAS5 could be regulated by P-STAT3 and affect resistance via miR-21/PDCD4 axis [125]. EDC4 could interact with RPA by alleviating DNA damage in cisplatin-resistant HeLa and SiHa cells [126]. IPO4-CEBPD-PRKDC axis is associated with chemoresistance by inhibiting PRKDC-driven DNA damage repair [127]. In addition, an increasing amount of noncoding RNAs have been confirmed and summarized with association to cisplatin resistance [128, 129]. For example, LncRNA HNF1A-AS1 could affect resistance by regulating miR-34b and promoting TUFT1 expression [130]. LncRNA OTUD6B-AS1 could mediate decreased regulation of miR-206 and increased expression of CCND2 [131]. LncRNA NNT-AS1 could improve chemoresistance via the miR-186/HMGB1 axis [132].

Combination chemotherapy with cisplatin and paclitaxel is a standard treatment in recurrent or advanced cervical cancer with an overall response rate of 29%–67% [133, 134]. Meanwhile, confirmed gain of 3q and loss of 11q chromosomes are early events in cancer progression. Subpopulations with differential responses to chemoradiotherapy may be selected into a single intrinsically resistant subpopulation after five weeks of the therapy [135]. Knockdown of Linc00511 could reduce paclitaxel resistance by regulating Bcl-2, MMP-2, MMP-9, MRP1, and P-GP expressions in HeLa cells [136].

![Fig. 5 Model of clonal progression of cervical cancer. Normal cervical cells may harbor genomic alterations and HPV integration after HPV infection. Some cells regress to normal spontaneously, while others round into clonally invasive carcinoma cells. Overwhelming majority cancer cells are removed or killed during conventional surgery and chemoradiotherapy. A few dormant or new subclones develop into recurrent, persist or metastatic cancer lesions. Systemic therapies (chemotherapy, radiotherapy, biotherapy and immunological therapy) can induce intrinsic or adapted resistant subclones. Resistant subclones contribute to uncontrolled disease and treatment failure.](image-url)
miR-214 under paclitaxel treatment could cause an increase in PARP and a decline in PI-3 kinase/Akt levels [137]. Circular RNA CircMYBL2 could enhance paclitaxel resistance by upregulating EGFR mediated by microRNA-665 in vitro and promoting tumor growth in vivo [138].

**Heterogeneity in terms of radiotherapy resistance**

Radiotherapy for cervical cancer is suitable for locally advanced and recurrent patients or other patients who can’t tolerate surgery. The majority of these patients own a worse prognosis due to advanced FIGO stage. The CCRT is the recommended treatment for advanced cervical cancer compared with radiotherapy alone, because CCRT increases patients’ local control rates and improves prognosis [139]. Integrated bioinformatics analysis on RNA sequencing has identified ten potential biomarkers related to radiotherapy resistance in cervical cancer. The results have indicated overexpression in tumor immune process pathways, including cellular defense response, negative regulation of the immunity, T cell and neutrophil activation, regulation of antigen presentation, and peptidyl-tyrosine autophosphorylation [140]. Other biomarkers, such as HIF-1 could enhance hypoxia-induced radio-resistance via targeting NDRG2 [141]. Overexpressed HOTAIR could promote HIF-1a and lead to radio-resistance in mice [142]. CD147 could induce resistance by regulating the percentage of G2/M phase cells and DNA double-strand breaks repair [143]. RhoC-ROCK2 involved DNA repair pathway is necessary for the radio-resistance phenotype in tumor cells [144]. SEPT9 could affect resistance by interacting with the HMGBl-R8 axis and mediating miR-375 [145]. Increased expression of HMBG3 correlated with hTERT could predict poor response to radiotherapy, advanced stage and worse outcome [146]. USP21 is overexpressed in radio-resistant patients and could activate the FOXM1/ Hippo signaling pathway [147]. Four specific miRNAs (miR-630, miR-1246, miR-1290, and miR-3138) could promote radio-resistance in vitro [148]. MiR-125 could modulate resistance through the downregulation of CDKN1A [149]. LncRNA UCA1 could promote radio-resistance associated glycolysis in SiHa and HeLa cells via HK2/glycolytic pathway [150]. LncRNA SNHG6 could enhance radio-resistant and promote cell growth via STYX/miR-485-3p axis [151]. Tumor radiotherapy has a certain impact on the TME, for instance, the generation of cancer-associated fibroblasts or macrophages [152, 153].

**Heterogeneity in terms of immunotherapy resistance**

After failing platinum-based chemotherapy, only about 10% of patients are responsive to additional cytotoxic agents. Immunotherapy of solid tumors is the research hotspot at present aiming to overcome immune suppression in TME and enhance tumor targeted immune attack. The main directions of immunotherapy include immune checkpoint inhibitors, therapeutic antibodies, therapeutic vaccines, cell therapy and small molecule inhibitors. Here we focus on the use of immune checkpoint inhibitors and therapeutic vaccines about the heterogeneity of cervical cancer.

Professors James P Allison and Tasuku Honjo won the 2018 Nobel Prize in Physiology or Medicine for discovering CTLA-4 and PD-1 as immune checkpoints and laying the foundation for tumor immunotherapy. The US Food and Drug Administration has already approved pembrolizumab for advanced cervical cancer patients with positive PD-L1. Clinical trials about the efficacy and safety of Pembrolizumab in advanced cervical cancer have been verified. Objective response rate (ORR) refers to the proportion of patients required for the reduction of the tumor to reach the expected value and to continue to the minimum expected time. ORR is commonly to be seen in evaluating the drug response in cancer patients undergoing clinical trials. The ORR of pembrolizumab in these patients has been increased to 14.6% [154]. Results of the phase III clinical trial of KEYNOTE-826 have expanded the indication for combined immunotherapy for persistent, recurrent or metastatic cervical cancer [155]. Results of the phase I/II clinical trial of CheckMate 358 (nivolumab) have shown an ORR of 26.3% with regardless of PD-L1 expression [156]. Three current trials of combining immunotherapy with chemotherapy for cervical cancer involved angiogenesis inhibitors and ICI combination therapy without conclusions (NCT03912415, NCT03635567, and NCT03556839) [157]. We summarized ongoing phase III clinical trials in cervical cancer and illustrated the effect targets for these therapies (Table 3 and Fig. 6). It can be seen from the current ongoing phase III clinical trials in cervical cancer that PD-1 inhibitors include Pembrolizumab, Camrelizumab, Cemiplimab, Prolgolimab (BCD-100), and QL-1604, while PD-L1 inhibitors include Durvalumab and Atezolizmab. Newly developed dual targeted drugs AK104 (PD-1 and CTLA-4 inhibitors) and SHR-1701 (PD-L1 and TGFβ inhibitors) have already been used in phase III clinical trials. The sensitivity of immunotherapy mainly depends on the heterogeneity of responses between tumor cells, immune-infiltrating cells, and other stroma cells in the TME. With the further development of scientific research, the refinement of immunotherapy indications marks the arrival of the era of precision therapy.

Novel immune checkpoints, for instance, TIGIT (T cell immune receptor with Ig and ITIM domains) have been
| Trial identifier | Brief title                                                                 | Estimated Enrollment | Criteria | Arms and Interventions                                                                 | Primary outcome measures [Time Frame] | Estimated Study Completion Date |
|------------------|-------------------------------------------------------------------------------|----------------------|----------|---------------------------------------------------------------------------------------|--------------------------------------|-------------------------------|
| NCT02422563      | Neoadjuvant chemotherapy followed by radical hysterectomy (op) versus primary chemo-radiation in cervical cancer FIGO stage IB2 and IIB | 534                  | IB2, IIB | Arm I: NACT + Radical hysterectomy Arm II: CCRT                                       | DFS [5 years]                        | October 2025                   |
| NCT02629718      | Neoadjuvant chemotherapy + surgery versus surgery in FIGO IB2 and IIA2 cervical cancer | 700                  | IB2, IIA2 | Arm I: NACT + Radical hysterectomy Arm II: Radical hysterectomy                         | DFS [2 years]                        | December 2022                  |
| NCT01101451      | Radiation therapy with or without chemotherapy in patients with stage I-IIA cervical cancer who previously underwent surgery | 360                  | I-IIA    | Arm I: EBRT/IMRT Arm II: Cisplatin + EBRT/IMRT                                        | RFS [11 years]                       | December 2021                  |
| NCT04723875      | Postoperative adjuvant chemotherapy in early-stage cervical cancer that not meet criteria of adjuvant therapeutic according to NCCN guideline | 306                  | IB1, IB2, IIA1 | Arm I: Chemotherapy Arm II: No intervention                                           | DFS [3 years]                        | January 2026                   |
| NCT05277688      | Adjuvant concurrent chemoradiotherapy versus radiotherapy in early-stage cervical cancer patients | 340                  | IA2-IIB  | Arm I: Cisplatin + IMRT Arm II: IMRT                                                 | RFS [5 years]                        | December 2027                  |
| NCT00980954      | Chemotherapy and pelvic radiation therapy with or without additional chemotherapy in treating patients with high-risk early-stage cervical cancer after radical hysterecytomy | 238                  | IA2-IIA  | Arm I: CCRT Arm II: CCRT + Chemotherapy                                               | DFS [4 years]                        | August 2026                    |
| NCT04989647      | Intermediate risk cervical cancer: radical surgery ± adjuvant radiotherapy    | 514                  | IB1-IIA  | Arm I: Surgery only Arm II: Surgery + Radiation Therapy                               | DFS [3 years]                        | December 2032                  |
| NCT03850866      | Study of durvalumab with chemoradiotherapy for women with locally advanced cervical cancer | 770                  | IB2 with positive nodes to IVA (FIGO2009) | Arm I: Durvalumab + CCRT Arm II: Placebo + CCRT                                       | PFS [4.5 years]                      | June 2023                      |
| Trial identifier | Brief title                                                                 | Estimated Enrollment | Criteria          | Arms and Interventions                                                                 | Primary outcome measures [Time Frame] | Estimated Study Completion Date |
|------------------|-----------------------------------------------------------------------------|----------------------|-------------------|----------------------------------------------------------------------------------------|--------------------------------------|-------------------------------|
| NCT04138992      | A study on the efficacy and safety of bevacizumab in untreated patients with locally advanced cervical cancer | 150 I-IIIC           | Arm I: Bevacizumab + NACT + CCRT Arm II: Bevacizumab + CCRT Arm III: CCRT | DFS [3 years]                           | May 2022                           |
| NCT02853604      | Study of ADXS11-001 in subjects with high risk locally advanced cervical cancer | 450 Locally advanced | Arm I: Placebo Arm II: ADXS11-001 | DFS [5 years]                            | October 2024                         |
| NCT01566240      | Induction chemotherapy plus chemoradiation as first line treatment for locally advanced cervical cancer | 500 IB1-IVA with positive lymph nodes | Arm I: CCRT Arm II: Chemotherapy + CCRT | OS [5 years]                           | May 2026                           |
| NCT03534713      | Induction chemotherapy followed by standard therapy in cervical cancer with aortic lymph node spread | 310 IB1-IVA with positive para-aortic lymph nodes | Arm I: NACT + CCRT Arm II: CCRT | OS [3 years]                           | December 2024                       |
| NCT03468010      | A trial comparing adjuvant chemotherapy with observation after concurrent chemoradiotherapy of cervical cancer (with pelvic or para-aortic node involvement) | 432 IB1-IVA with positive lymph nodes | Arm I: CCRT Arm II: Chemotherapy + CCRT | PFS [3 years]                          | March 2025                          |
| NCT05173272      | Induction chemotherapy followed by concurrent chemoradiation in advanced cervical cancer | 286 IB3-IIIB          | Arm I: NACT + CCRT Arm II: CCRT | PFS [3 years]                           | February 2028                        |
| NCT04974346      | Para-aortic prophylactic irradiation for locally advanced cervical cancer | 450 IB2-IVA with positive pelvic lymph nodes and negative common iliac and paraaortic lymph nodes (FIGO 2009) | Arm I: Para-aortic Prophylactic Irradiation + CCRT Arm II: CCRT | PFS [3 years]                          | August 2030                        |
| NCT05235516      | A study of AK104/placebo combined with chemoradiotherapy for the treatment of locally advanced cervical cancer | 636 IIIA-IVA          | Arm I: AK104 + CCRT Arm II: Placebo + CCRT | PFS [4.5 years]                         | May 2029                           |
| NCT01414608      | Cisplatin and radiation therapy with or without carboplatin and paclitaxel in patients with locally advanced cervical cancer | 900 IB1 with node positive, IB2, IIa, IIb, or IVA (FIGO 2008) | Arm I: CCRT Arm II: CCRT + Chemotherapy | OS [5 years]                           | July 2022                          |
### Table 3 (continued)

| Trial identifier | Brief title | Estimated Enrollment | Criteria | Arms and Interventions | Primary outcome measures (Time Frame) | Estimated Study Completion Date |
|------------------|-------------|----------------------|----------|------------------------|----------------------------------------|-------------------------------|
| NCT05189028     | Study of neoadjuvant chemotherapy versus definite concurrent chemoradiotherapy for locally advanced bulk cervical cancer | 290 | IB3, II[A2, IIB-IVA | Arm I: NACT Arm II: CCRT | OS [2 years] | June 2025 |
| NCT04221945     | Study of chemoradiotherapy with or without pembrolizumab (MK-3475) for the treatment of locally advanced cervical cancer | 980 | IB2-IVA with positive nodes (FIGO 2014) | Arm I: CCRT + Pembrolizumab Arm II: CCRT + Placebo | PFS [38 months] OS [46 months] | December 2024 |
| NCT03635567     | Efficacy and safety study of first-line treatment with pembrolizumab (MK-3475) plus chemotherapy versus placebo plus chemotherapy in women with persistent, recurrent, or metastatic cervical cancer | 600 | Persistent, recurrent, metastatic | Arm I: Pembrolizumab + Chemotherapy ± Bevacizumab Arm II: Placebo + Chemotherapy ± Bevacizumab | PFS [2 years] OS [2 years] | November 2022 |
| NCT04906993     | Camrelizumab combined with famitinib malate for treatment of recurrent/metastatic cervical cancer | 440 | Recurrent, metastatic | Arm I: Camrelizumab + Famlitinib malate + Chemotherapy Arm II: Chemotherapy | PFS [2 years] OS [2 years] | May 2023 |
| NCT04733820     | Clinical efficacy of adjuvant chemotherapy in patients with locally advanced cervical cancer who did not meet the NCCN guidelines for adjuvant treatment after NACT combined with surgery | 340 | IB3-IIIB | Arm I: Chemotherapy Arm II: No intervention | DFS [5 years] | February 2028 |
| NCT05367206     | Neoadjuvant chemotherapy followed by chemoradiation versus chemoradiation for stage IIIC cervical cancer patients: a randomized phase III trial | 280 | IIIC | Arm I: albumin-bound paclitaxel and carboplatin + CCRT Arm II: CCRT | PFS [3 years] | March 2027 |
| NCT03556839     | Platinum chemotherapy plus paclitaxel with bevacizumab and atezolizumab in metastatic carcinoma of the cervix | 404 | IVB, persistent, recurrent | Arm I: Chemotherapy + Bevacizumab Arm II: Atezolizumab + Chemotherapy + Bevacizumab | PFS [48 months] OS [48 months] | December 2023 |
utilized combined with anti-PD-1 antibody in recurrent or metastatic cervical cancer (NCT04693234). The application of immune checkpoint inhibitors is limited by the heterogeneity of checkpoint expression on tumor cell surface and immune-activated state in TME. Decreased tumor associated lymphocytes and retained HPV E6/E7 gene expressions may promote treatment resistance during chemoradiation therapy in locally advanced cervical cancer.
cervical cancer patients [158]. Oncogenic E5, E6, and E7 proteins encoded by HR-HPV, especially HPV16 and 18, are implicated in the PD1/PD-L1 pathway leading to increased PD-L1 expression [159–161]. B cells are activated by radiation combined with PD-1 blockade and could improve overall survival in HPV-associated squamous cell carcinomas patients [162]. LSD1 inhibitor combined with anti-CD47/PD-L1 monoclonal antibodies could more effectively inhibit tumor growth in a subcutaneous xenograft model because of increasing the expressions of CD47 and PD-L1 [163]. Other driver genes (PI3KCA, PI3KCB, DVL3, WWTR1 and ERBB2) in regulating immune response or immune cell infiltration are with application prospect [164]. Three single-nucleotide polymorphisms (SNPs), specifically PAX8, CLPTM1L, and HLA genes, are replicated in cervical cancer patients and are associated with cervical carcinogenesis through disruption in apoptotic and immune response pathways [165, 166].

Therapeutic vaccines have also shown some success in patients with advanced cervical cancer. An alphavirus-based treatment vaccine combined with sunitinib and irradiation could elicit superior antitumor effects [167]. HPV recombinant vaccine prime-boost could enhance CD8+ T cell mediated tumor cytotoxicity [168]. PD-1 blockade combined with intra-tumoral therapeutic vaccination could elicit HPV16-associated tumor regression in a murine model [169]. The combined application of cervical cancer therapeutic vaccine and immunotherapy has become the general trend at present [170].

Inferring heterogeneity with histological diversity

The histological diversity of cervical cancer is also a manifestation of tumor heterogeneity. There were significant differences in treatment sensitivity and prognosis among different histological types. In the previous paragraphs, we have mainly discussed the characteristics of cervical squamous cell carcinoma, while in this section we will focus on the molecular and clinical characteristics of cervical cancer stem cells, cervical adenocarcinoma cells, and cervical neuroendocrine cell subtypes.

Heterogeneity of cervical cancer stem cell

The clonal evolution model and the cancer stem cell (CSC) model have been used to illustrate intra-tumor
heterogeneity. In the first model, stochastic mutations in individual tumor cells form in the tumor microenvironment, the superior sub-clonal cells dominate and proliferate under adaptation and selection [171]. Another model highlights the cellular plasticity and mutational differentiation hierarchy formation generated by CSC clones [172, 173]. We attempt to interpret cervical cancer heterogeneity by describing the cell surface biomarkers, molecular mechanism of stem cell regulation and differences in cytological behavior as follows.

Cervical cancer stem-like cells (CCSC) with an expression pattern of CD44 (+)CD24(-) surface biomarkers have been isolated from HeLa and SiHa cell lines which present higher capabilities in cell growth, self-renew, chemotherapeutic drug and radiation therapy resistance [174, 175]. Another prolonged Trichostatin A-selected HeLa cell expressing Sox2(+)/Oct4(+)/Nanog(+) markers display enhanced migration, invasion, and malignancy abilities both in vitro and in vivo, which can also be regulated by STAT3 [176–178]. Ubiquitin B has been confirmed as a key gene in the maintenance of Sox2(+)/Oct4(+)/Nanog(+) CCSC [177]. Hiwi and Gremlin 1 can be regarded as cervical CSC markers because the increased gene expressions facilitate in vitro tumor sphere formation and in vivo tumorigenicity [179, 180]. The extended phenotype of CCSC has been determined with CK-17, p63 +, All +, CD49f + and higher Aldehyde dehydrogenase activity [181]. Besides, the Wnt/beta-catenin pathway is essential to maintain tumorigenicity by microRNA-135a induced CD133(+) CCSC and CCSC related transcription factor levels promoted by LGR5. Wnt3a stimulation may increase tumor size and self-renew [182, 183]. Cancer is a result of uncontrolled cell growth caused by mutations or epigenetic alterations, while cancer stem cell heterogeneity contributes to the whole process of tumorigenesis, recurrence, metastasis and treatment resistance.

Heterogeneity of cervical adenocarcinoma
Cervical adenocarcinomas comprise approximately 25% of cervical cancer in the USA with higher histologically heterogeneous compared to squamous cell carcinoma [184]. The World Health Organization (WHO) classification and a more innovative International Endocervical Criteria and Classification (IECC) are commonly recognized classification criteria [185, 186]. The traditional WHO 2014 system divides cervical adenocarcinomas into serous, mucinous, endometrioid, clear cell and some other types based on pathological features. The IECC 2018 system attempts to subdivide adenocarcinomas into HPV-associated (HPVA) and non-HPV-associated (NHPVA) categories [187]. NHPVAs, in particular gastric type is significantly associated with age, horizontal extent, invasive depth or lympho-vascular invasion, advanced stage, worse disease-free survival (DFS) or disease-specific survival (DSS). Among the HPVAs, invasive stratified mucin producing carcinoma subtypes have shown worse DFS and DSS [188]. According to the revised WHO classification 2020, 92.7% of HPVAs can be recognized by the presence of luminal mitoses and apoptosis in addition to mucinous adenocarcinomas [189, 190]. Distinct molecular profiles have been demonstrated between SCC and adenocarcinoma as mentioned above, which suggests that more tailored treatment strategies are necessary [81]. Gastric-type cervical adenocarcinoma has been detected with somatic mutations in TP53, KRAS, CDKN2A, and STK11. Prevalent mutations of PIK3CA and PTEN enriched in the PI3K/Akt/mTOR signaling pathway has also been identified [191]. Potentially driven mutations have been identified in BRAF, ERBB2 and ERBB3. Copy-number aberrations (CNAs) are deletions or expansions of chromosomes/gens in somatic cells. Low levels of CNAs without recurrent amplifications or homozygous deletions are also confirmed [192]. Further similarities and differences genetic heterogeneity between HPVA and HPV-positive squamous cell carcinoma remain to be further studied.

Heterogeneity of other rare histological cervical cancer
Neuroendocrine carcinoma of the cervix (NECC) is a variant of CC with accounts for 1–1.5% [193]. A large meta-analysis with 3538 NECC cases has identified a mean recurrence-free survival of 16 months and overall survival of 40 months [194]. The small cell cervical carcinoma (SCCC) is the most common type of NECC with median overall survival ranging between 10–13 months in advanced SCCC [133]. Adjuvant chemotherapy or chemoradiation is associated with higher five-year survival in 188 SCCC patients [195]. FIGO stage is the unique prognostic factor impacting both overall survival and DFS in a multiple retrospective study with 93 SCCC patients [196]. SCCC is specifically associated with HPV18 infection and its genetic alterations are regulated through PI3K/MSK/mTOR, MAPK, and TP53/BRCA pathways [197]. Driven mutation genes KRAS, PIK3CA, IRS2, SOX2 and homogeneous recombination repair genes are potential therapeutic targets [198].

Conclusions and future perspectives
HPV-associated cervical cancer is a kind of heterogeneous malignant tumor from many perspectives, and its treatment in the advanced stage is extremely difficult. HPV and drug therapy are two extrinsic factors that are closely related to the heterogeneity of cervical cancer. Meanwhile, an in-depth understanding of tumor heterogeneity is a critical issue in developing precision
treatment and screening strategies. Our understanding of the molecular and phenotypic heterogeneity in cervical cancer has improved and benefited from the development of deep sequencing and single cell sequencing technology. Nevertheless, it will take time to get breakthrough results on the heterogeneity of tumor microenvironment and treatment responses in advanced cancer. The integration of genomic, transcriptomic and epigenetic information that captures intra-tumoral heterogeneity will reveal the panoramic view of tumor regulatory mechanisms and will promote breakthroughs in precision medicine.

Abbreviations

CC: Cervical cancer, CCEMC: Cervical Cancer Elimination Modelling Consortium, CCRT: Concurrent chemotherapy, CCSC: Cervical cancer stem-like cell, ceRNA: Competing endogenous RNA, CIN: Intraepithelial neoplasia, circRNA: Circular RNA, CNA: Copy number alteration, COSMIC: Catalogue Of Somatic Mutations In Cancer, CSC: Cancer stem cell, CTL: Cytotoxic T lymphocyte, DEG: Differential expressed gene, DFS: Disease-free survival, DSS: Disease-specific survival, ECM: Extracellular matrix, FIGO: International Federation of Gynecology and Obstetrics, HPV: Human papillomavirus, HPVAs: HPV-associated, HR: HPV: High-risk HPV, ICC: Invasive cervical cancer, IEC: International Endocervical Criteria and Classification, IHC: Immunohistochemistry, LMICs: Low-income and middle-income countries, IncRNA: Long non-coding RNA, miRNA: Micro-RNA, MLPA: Multiplex ligation probe amplification, NACT: Neoadjuvant chemotherapy, NECC: Neuroendocrine carcinoma of the cervix, NGS: Next-generation sequencing, NHPVA: Non-HPV-associated, OCP: Oropharyngeal cancer, ORR: Objective response rate, PD-L1: Programmed death-ligand 1, PDL2: Programmed death-ligand 2, PIM: Post infection microenvironment, RHP: Recurrent heterogeneous program, RT-PCR: Reverse transcription-polymerase chain reaction, SBS: Single-base substitution, SCC: Squamous cell carcinoma, SCCC: Small cell cervical carcinoma, scRNA-seq: Single-cell RNA sequencing, sSNV: Somatic single nucleotide variant, TAM: Tumor-related macrophage, Th1: T-helper 1, TIGIT: T cell immune receptor with Ig and ITIM domains, TME: Tumor microenvironment, WGS: Whole-genome sequencing, WHO: World Health Organization.

Acknowledgements

Not applicable.

Authors’ contributions

ZQH and QS conceived the manuscript. QS and LLW collected relevant references, drafted the manuscript, and finished the figures and tables. CZ, ZYH and ZQH offered crucial content revision and language polishing. ZQH completed the final manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by the National Natural Science Foundation of China (81974414, 81772788, 81873430).

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Pett M, Coleman N. Integration of high-risk human papillomavirus: a key event in cervical carcinogenesis? J Pathol. 2007;212(4):356–67.
2. Castellsague X. Natural history and epidemiology of HPV infection and cervical cancer. Gynecol Oncol. 2008;110(3 Suppl 2):54–7.
3. Marusyk A, Janiszewska M, Polyak K. Intraatumor Heterogeneity: The Rosetta Stone of Therapy Resistance. Cancer Cell. 2020;37(4):471–84.
4. Cook O. The Mendelian Inheritance of Mutations. Science. 1990;28(707):86–8.
5. Williams MJ, Werner B, Barnes CP, Graham TA, Sottonvia A. Identification of neutral tumor evolution across cancer types. Nat Genet. 2016;48(3):238–44.
6. McGranahan N, Swanton C. Biological and therapeutic impact of intra-tumor heterogeneity in cancer evolution. Cancer Cell. 2015;27(1):15–26.
7. Alizadeh AA, Aranda V, Bardelli A, Blanpain C, Bock C, Borowski C, Caldas C, Califano A, Doherty M, Elsner M, et al. Toward understanding and exploiting tumor heterogeneity. Nat Med. 2015;21(8):846–53.
8. Almond V, Marusyk A, Polyak K. Cellular heterogeneity and molecular evolution in cancer. Annu Rev Pathol. 2013;8:277–302.
9. Fan J, Slowikowski K, Zhang F. Single-cell transcriptomics in cancer: computational challenges and opportunities. Exp Mol Med. 2020;52(9):1452–63.
10. Muller S, Diaz A. Single-Cell mRNA Sequencing in Cancer Research: Integrating the Genomic Fingerprint. Front Genet. 2017;8:73.
11. Isayeva T, Li Y, Massuah D, Brandwein-Gensler M. Human papillomavirus in non-oropharyngeal head and neck cancers: a systematic literature review. Head Neck Pathol. 2012;6 Suppl 1(Suppl 1):S104-120.
12. Szymonowicz KA, Chen J. Biological and clinical aspects of HPV-related cancers. Cancer Biol Med. 2020;17(4):864–78.
13. Johnson DE, Burtensh L, Leemans CR, Lui WY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Primers. 2020;6(1):92.
14. Sun H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020. GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49.
15. Arbyn M, Weiderpass E, Bruni L, de Sanjose S, Saraiya M, Ferlay J, Bray F. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob Health. 2020;8(2):e191–203.
16. Bruni L, Diaz M, Barrionuevo-Rosas L, Herrero R, Bray F, Bosch FX, de Sanjose S, Castellsague X. Global estimates of human papillomavirus vaccination coverage by region and income level: a pooled analysis. Lancet Glob Health. 2016;4(7):e453-63.
17. Simms KT, Steinberg J, Caruana M, Smith MA, Lew JB, Soerjomataram I, Castle PE, Bray F, Canfell K. Impact of scaled up human papillomavirus vaccination and cervical screening and the potential for global elimination of cervical cancer in 181 countries, 2020–99: a modelling study. Lancet Oncol. 2019;20(3):394–407.
18. Canfell K, Kim JJ, Brisson M, Keane A, Simms KT, Caruana M, Burger EA, Martin D, Nguyen DTN, Benard E, et al. Mortality impact of achieving WHO cervical cancer elimination targets: a comparative modelling analysis in 78 low-income and lower-middle-income countries. Lancet. 2020;395(10224):591–603.
19. Zhou Z, Fairley CK, Ong JH, Hocking J, Canfell K, Ma X, Chow EPF, Xu X, Zhang L, Zhuang G. Domestic HPV vaccine price and economic returns for cervical cancer prevention in China: a cost-effectiveness analysis. Lancet Glob Health. 2020;8(10):e1335–44.
20. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders Pj, Meijer CJ. International Agency for Research on Cancer Multicenter Cervical Cancer Study G. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003;348(6):518–27.
21. Laghedn C, Eklund C, Lamin H, Klope SN, Lei J, Efstrom KM, Sundstrom K, Andrae B, Sparen P, Dillner J. Nationwide comprehensive human papillomavirus (HPV) genotyping of invasive cervical cancer. Br J Cancer. 2018;18(10):1377–81.

22. Guan P, Howell-Jones R, Li N, Bruni L, de Sanjose S, Franceschi S, Clift GM. 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.

23. Clift GM, Tully S, Franceschi S. Carcinogenicity of Human Papilloma virus (HPV) Types in HIV-Positive Women: A Meta-Analysis From HPV Infection to Cervical Cancer. Clin Infect Dis. 2017;64(9):1228–35.

24. Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, de Sanjose S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis. 2010;202(2):1789–99.

25. Soderlund-Strand A, Uhnoo I, Dillner J. Change in population prevalence of human papillomavirus after initiation of vaccination: the high-throughput HPV monitoring study. Cancer Epidemiol Biomarkers Prev. 2014;23(12):2757–64.

26. Sekine M, Yamaguchi M, Kudo R, J B Hanley S, Hara M, Adachi S, Ueda Y, Miyagi E, Ikeda S, Yagi A, et al. Epidemiologic Profile of Type-Specific Human Papillomavirus Infection after Initiation of HPV Vaccination. Vaccines (Basel). 2020;8(3):425.

27. Woodman CB, Collins SI, Young LS. The natural history of cervical HPV infection: unsettled issues. Nat Rev Cancer. 2007;7(11):1–22.

28. Da Silva MLR, De Albuquerque B, Allyrio T, De Almeida VD, Cobucci RNO, Bezerra FL, Andrade VS, Lanza DCF, De Azevedo JCV, De Araujo JMG, et al. The role of HPV-induced epigenetic changes in cervical carcinogenesis (Review). Biomed Rep. 2021;15(1):60.

29. McDermott AA. Human papillomaviruses: diversity, infection and host interactions. Nat Rev Microbiol. 2022;20(2):95–108.

30. Jiang B, Xue M. Correlation of E6 and E7 levels in high-risk HPV16 type infected cervical lesions with CCL20 and Langerhans cells. Genet Mol Res. 2015;14(3):10473–81.

31. Yuan Y, Cai X, Shen F, Ma F. HPV post-infection microenvironment and carcinogenesis (Review). Biomed Rep. 2021;15(1):60.

32. Zhang R, Shen C, Zhao L, Wang J, McCrae M, Chen X, Lu F. Dysregulation of host cellular genes targeted by human papillomavirus (HPV) integration contributes to HPV-related cervical carcinogenesis. Int J Cancer. 2020;146(4):1077–86.

33. Holmes A, Lameiras S, Deloger M, Morel A, Vacher S, Lecerf C, Bodelon C, Vinokurova S, Sampson JN, den Boon JA, Walker JL, Hopman AH, Theelen W, Hommelberg PP, Kamps MA, Herrington CS, Morrisson LE, Speel EJ, Smetsd F, Ramaekers FC. Genomic integration of oncogenic HPV and gain of the human telomerase gene TERC at 3q26 are strongly associated events in the progression of uterine cervical dysplasia to invasive cancer. J Pathol. 2006;206(4):412–9.

34. Schmitz M, Driesch C, Jansen L, Runnebaum IB, Durst M. Non-random integration of the HPV genome in cervical cancer. J Pathol. 2006;206(4):412–9.

35. Schmitz M, Driesch C, Jansen L, Runnebaum IB, Durst M. Non-random integration of the HPV genome in cervical cancer. J Pathol. 2006;206(4):412–9.

36. Morrison BI, Steel JC, Morris JC. Reduction of MHC-I expression limits T-lymphocyte-mediated killing of Cancer-initiating cells. BMC Cancer. 2016;18(1):469.

37. Gray J, Westerhof LM, MacLeod MKL. The roles of resident, central and effector memory CD4 T-cells in protective immunity following infection or vaccination. Immunology. 2018;154(4):574–81.

38. Yuan Y, Cai X, Shen F, Ma F. HPV post-infection microenvironment and cervical cancer. Cancer Lett. 2021;497:243–54.

39. Kawanishi S, Ohnishi S, Ma N, Hiraku Y, Murata M. Crosstalk between DNA Damage and Inflammation in the Multiple Steps of Carcinogenesis. Int J Mol Sci. 2017;18(8):1808.

40. Zou D, Ye M, Zhang W. E6/E7 oncoproteins of high risk HPV-16 upregulate M1-MIP-1β and MIP-1β and promote the migration of cervical cancer cells. Int J Clin Exp Pathol. 2018;11(3):1637–44.

41. Longworth MS, Laimins LA. Pathogenesis of human papillomavirus in differentiating epithelia. Microbiol Mol Biol Rev. 2004;68(2):362–72.

42. Chen JJ. Genomic Instability Induced By Human Papillomavirus Onconogenes. N Am J Med Sci. 2013;3(2):43–7.

43. Iqbal L, Anazawa H, Furumaya R, Ikawaki R, Nakayama K, Kinoshita K, Tanaka K. High levels of chromosomal instability facilitate the tumor growth and sphere formation. Cancer Sci. 2022;113(11):2727–37.

44. El Awdy MK, Kaplan JB, O'Brien SJ, Burt RD. Molecular analysis of integrated human papillomavirus 16 sequences in the cervical cancer cell line SiHa. Virology. 1987;159(2):389–98.

45. Cooper K, Herrington CS, Graham AK, Evans MF, McGee JO. In situ evidence for HPV 16, 18, 31 and 33 integration in cervical squamous cell cancer in Britain and South Africa. J Clin Pathol. 1991;44(5):406–9.

46. Huang J, Qian Z, Gong Y, Wang Y, Guan Y, Han Y, X, Huang W, Ji L, Xu J, et al. Comprehensive genomic variation profiling of cervical intraepithelial neoplasia and cervical cancer identifies potential targets for cervical cancer early warning. J Med Genet. 2019;56(3):186–94.
91. Zhu MY, Chen F, Nyaz M, Sui S, Gao DM. Variation in apoptotic gene expression in cervical cancer through oligonucleotide microarray profiling. J Low Genit Tract Dis. 2015;19(1):46–54.
92. Liu MY, Zhang H, Hu YJ, Chen YW, Zhao XN. Identification of key genes associated with cervical cancer by comprehensive analysis of transcriptome microarray and methylation microarray. Oncol Lett. 2016;12(1):473–8.
93. Wang S, Chen X. Identification of potential biomarkers in cervical cancer with combined public mRNA and miRNA expression microarray data analysis. Oncol Lett. 2018;16(4):5200–8.
94. Moussavi SZ, Farrokhzadeh V, Mohktari-Azad T, Shahnahmoodi S, Farahmand M, Farzanehpoor M, Jallalvand S. The dysregulation of microarray gene expression in cervical cancer is associated with overexpression of a unique messenger RNA signature. Iran J Microbiol. 2020;12(6):629–35.
95. Peng G, Dan W, Jun W, Junjun Y, Tong R, Baoli Z, Yang X. Transcriptome profiling of the cancer and adjacent nontumor tissues from cervical squamous cell carcinoma patients by RNA sequencing. Tumour Biol. 2015;36(6):3309–17.
96. Wang Y, Liu L, Chen Z. Transcriptome profiling of cervical cancer cells acquired resistance to cisplatin by deep sequencing. Artif Cells Nanomed Biotechnol. 2019;47(1):2820–9.
97. Chen T, Yang S, Xu J, Lu W, Wei X. Transcriptome sequencing profiles of cervical cancer tissues and SiHa cells. Funct Integr Genomics. 2020;20(2):211–21.
98. Kinker GS, Greenwald AC, Tal R, Orlova Z, Cuoco MS, McFarland JM, et al. Single-cell transcriptomics reveals the landscape of intra-tumoral heterogeneity and transcriptomic activities of EC in CC. Mol Ther Nucleic Acids. 2021;24:682–94.
99. Gu M, He T, Yuan Y, Duan S, Li X, Shen C. Single-Cell RNA Sequencing Reveals Multiple Pathways and the Tumor Microenvironment Could Lead to Chemotherapy Resistance in Cervical Cancer. Front Oncol. 2021;11:753386.
100. Gagliardi A, Porter VL, Zong Z, Bowby R, Tittmuss E, Namirembe C, Griner NB, Petrello H, Bowen J, Chan SK, et al. Analysis of Ugandan cervical carcinomas identifies human papillomavirus clade-specific epi-genome and transcriptome landscapes. Nat Genet. 2020;52(1):1208–18.
101. Li C, Guo L, Li S, Hua K. Single-cell transcriptomics reveals the landscape of intra-tumoral heterogeneity and transcriptomic activities of EC in CC. Mol Ther Nucleic Acids. 2021;24:682–94.
102. Burk TK. Novel mutations in cervical cancer. Lancet Oncol. 2017;18(3):e137.
103. Ren T, Sou J, Liu J, Wang S, Shu S, Xiang Y, Lang HJ. Using low-coverage whole genome sequencing technique to analyze the chromosomal copy number alterations in the exfoliative cells of cervical cancer. J Gynecol Oncol. 2018;29(5):e78.
104. Lai EC. Micro RNAs are complementary to 3’ UTR sequence motifs that mediate negative post-transcriptional regulation. Nat Genet. 2002;30(4):353–4.
105. Ulltysky J, Bartel DP. lincRNAs: genomics, evolution, and mechanisms. Cell. 2013;154(1):26–46.
106. Haddad G, Lorenzen JM. Biogenesis and Function of Circular RNAs in Cancer. Genes. 2019;10:4028.
107. Kartha RV, Subramanian S. Competing endogenous RNAs (ceRNAs): new entrants to the intricacies of gene regulation. Front Genet. 2014;5:8.
108. Basera A, Hull R, Demetriou D, Bates DO, Kaufmann AM, Dlamini Z, et al. Dysregulation of lncRNA HNF1A-AS1 affects cisplatin resistance in cervical cancer cells. Tumour Biol. 2019;40(11):117499.
109. Ferrandina G, Lauriola L, Distefano MG, Zannoni GF, Gessi M, Legge F, Maggiano N, Mancuso S, Capella A, Scambia G, et al. Increased cyclooxygenase-2 expression is associated with chemotherapy resistance and poor survival in cervical cancer patients. J Clin Oncol. 2020;40(4):973–81.
110. Cai L, Wang Z, Liu D. Interference with endogenous EZH2 reverses the chemotherapy drug resistance in cervical cancer cells partly by up-regulating Dicer expression. Tumour Biol. 2016;37(5):6359–69.
111. Duan W, Liu X. PSAT1 Upregulation Contributes to Cell Growth and Cisplatin Resistance in Cervical Cancer Cells via Regulating PI3K/AKT Signaling Pathway. Oncol Lett. 2020;20(1):512–8.
112. Wang N, Hou MS, Zhan Y, Shen XB, Xue HY. MALAT1 promotes cisplatin resistance in cervical cancer by activating the PI3K/AKT pathway. Eur Rev Med Pharmacol Sci. 2021;25(22):7653–9.
113. Tang Q, Chen Z, Zhao L, Xu H. Circular RNA hsa_circ_0000515 acts as a miR-326 sponge to promote cervical cancer progression through up-regulation of ELK1. Aging (Albany NY). 2019;11(2):9982–99.
114. Eddy GL, Bundy BN, Creasman WT, Spirtos NM, Mannel RS, Hannigan E, O’Connor D. Treatment of ‘bulky’ stage III 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.
115. Duenas-Gonzalez A, Zarba JJ, Patel F, Alcedo JC, Besilja S, Casanova L, et al. Identification of key biomarkers in cervical tumors. In Advanced Cervical Cancer: NRG Oncology-Gynecologic Oncology Group Study 240 (NCT 00830362). Mol Cancer Ther. 2020;19(11):2363–70.
116. Pfandeler KS, Tewari KS. Changings paradigms in the systemic treatment of advanced cervical cancer. Am J Obstet Gynecol. 2016;214(1):22–30.
117. Koh WJ, Abu-Rustum NR, Bean S, Bradley K, Campos SM, Cho KR, Chen HS, Chu C, Clark R, Cohn D, et al. Cervical Cancer, Version 3.2019, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2019;17(1):64–84.
118. Zhu H, Luo H, Zhang W, Shen Z, Hu X, Zhu X. Molecular mechanisms of cisplatin resistance in cervical cancer. Drug Des Devel Ther. 2016;10:1885–95.
119. Ferrandina G, Lauriola L, Distefano MG, Zannoni GF, Gessi M, Legge F, Maggiano N, Mancuso S, Capella A, Scambia G, et al. Increased cyclooxygenase-2 expression is associated with chemotherapy resistance and poor survival in cervical cancer patients. J Clin Oncol. 2020;40(4):973–81.
1110. Kina S, Kinjo T, Liang F, Nakahone Y, Yamamoto H, Arasaki A. Targeting Epha4 abrogates intrinsic resistance to chemotherapy in well-differentiated cervical cancer cell line. Eur J Pharmacol. 2018;840:70–8.
1111. Cai L, Wang Z, Liu D. interference with endogenous EZH2 reverses the chemotherapy drug resistance in cervical cancer cells partly by up-regulating Dicer expression. Tumour Biol. 2016;37(5):6359–69.
1112. Duan W, Liu X. PSAT1 Upregulation Contributes to Cell Growth and Cisplatin Resistance in Cervical Cancer Cells via Regulating PI3K/AKT Signaling Pathway. Oncol Lett. 2020;20(1):512–8.
1113. Wang N, Hou MS, Zhan Y, Shen XB, Xue HY. MALAT1 promotes cisplatin resistance in cervical cancer by activating the PI3K/AKT pathway. Eur Rev Med Pharmacol Sci. 2018;22(22):7653–9.
1114. Fang X, Zhong G, Wang Y, Lin Z, Lin R, Yao T. Low GASS expression may predict poor survival and cisplatin resistance in cervical cancer. Cell Death Dis. 2020;11(7):531.
1115. Wu X, Zhong Y, Chen Q, Zhang X, Zhang H. Enhancer of miRNA Decapping protein 4 (EDC4) interacts with replication protein a (RPA) and contributes to Cisplatin resistance in cervical cancer by alleviating DNA damage. Hereditas. 2020;157(1):41.
1116. Zhou Y, Liu F, Xu Q, Yang B, Li X, Jiang S, Hu L, Zhang X, Liu L, Qi et al. Inhibiting Importin 4-mediated nuclear import of CBP/P300 enhances chemosensitivity by repression of PRKDC-driven DNA damage repair in cervical cancer. Oncogene. 2020;39 (34):5633–48.
1117. Wen X, Liu S, Sheng J, Cui M. Recent advances in the contribution of noncoding RNAs to cisplatin resistance in cervical cancer. Peeri. 2020;8:e9234.
1118. Masadrah R, Rauf S, Pratama MY, Timbelli C, Pascut D. The Role of micro-RNAs in the Cisplatin- and Radio-Resistance of Cervical Cancers (Basel). 2021;13(1):1168.
1119. Luo X, Wei J, Yang FL, Pang XX, Shi F, Wen YX, Liao BY, Wang JL. Exosomal IncRNA HNF1A-AS1 affects cisplatin resistance in cervical cancer cells through regulating microRNA-34b/422A1 axis. Cancer Cell Int. 2019;19(3):31.
1120. Hou H, Yu R, Zhao H, Yang H, Hu Y, Hu Y, Guo J. LncRNA OTUD6B-AS1 Induces Cisplatin Resistance in Cervical Cancer Cells Through Up-Regulating OCT3/4. J Gynecol Oncol. 2019;30(6):e95.
1121. Liu Y, Guo R, Qiao Y, Han L, Liu M. LncRNA NNT-AS1 contributes to the cisplatin resistance of cervical cancer through NNT-AS1/miR-186/ HMGB1 axis. Cancer Cell Int. 2020;20:190.
Lorusso D, Petrelli F, Coinu A, Raspagliesi F, Baris S. A systematic review comparing cisplatin and carboplatin plus paclitaxel-based chemotherapy for recurrent or metastatic cervical cancer. Gynecol Oncol. 2014;133(1):17–23.

Seol HJ, Utkin R, Ki KD, Lee JM. Cytoxic and targeted systemic therapy in advanced and recurrent cervical cancer: experience from clinical trials. Tohoku J Exp Med. 2014;232(4):269–76.

Cooke SL, Temple J, Macarthur S, Zahra MA, Tan LT, Crawford RA, Ng CK, Jimenez-Linan M, Sala E, Brenton JD. Intra-tumour genetic heterogeneity and poor chemoradiation response in cervical cancer. Br J Cancer. 2011;104(2):361–8.

Mao BD, Xu P, Xu P, Zhong Y, Ding WW, Meng QZ. LINC00511 knockdown prevents cervical cancer cell proliferation and reduces resistance to paclitaxel. J Biosci. 2019;44(2):1.

Sen P, Ghosal S, Hazra R, Mohanty R, Arega S, Sahu B, Ganguly N. CRISPR-mediated knockdown of miR-214 modulates cell fate in response to anti-cancer drugs in HPV-negative and HPV-positive cervical cancer cells. J Biosci. 2020;45:80.

Dong M, Li P, Xie Y, Wang Z, Wang R. CircMYBL2 regulates the resistance of cervical cancer cells to paclitaxel via miR-665-dependent regulation of EGFR. Drug Dev Res. 2021;82(8):1193–205.

Rose PG, Bundy BN, Watkins EB, Thigpen JT, Deppe G, Maiman MA, Liu J, Zhang J, Wang X, Li Y, Chen Y, Li K, Zhang J, Yao L, Guo G. HIF-1 down prevents cervical cancer cell proliferation and reduces resistance to chemotherapy for locally advanced cervical cancer. N Engl J Med. 1999;340(15):1144–53.

Feng Y, Wang Z, Yang N, Liu S, Yan J, Song J, Yang S, Zhang Y. Identification of Biomarkers for Cervical Cancer Radiotherapy Resistance Based on RNA Sequencing Data. Front Cell Dev Biol. 2021;9:3873.

Liu J, Zhang J, Wang X, Li Y, Chen Y, Li K, Zhang J, Yao L, Guo G. HIF-1 and NDRG2 contribute to hypoxia-induced radiosensitivity of cervical cancer Hela cells. Exp Cell Res. 2010;316(2):1985–93.

Li N, Meng DQ, Gao L, Xu Y, Liu PJ, Tian YW, Li ZY, Zhang Y, Tie XJ, Xu ZQ. Overexpression of HOTAIR leads to radiosensitivity of human cervical cancer via promoting HIF-1α expression. Radiat Oncol. 2018;13(1):210.

Tu X, Liang S, Zhu J, Ke G, He H, Wu X. Extracellular matrix metalloproteinase inducer (CD147/BSG/EMMPRIN)-induced radiosensitivity in cervical cancer by regulating the percentage of the cells in the G2/m phase of the cell cycle and the repair of DNA Double-strand Breaks (DSBs). Am J Transl Res. 2016;8(6):2498–511.

Pranatharthi A, Thomas P, Udhayakrishnan A, Bhavani C, Suresh SB, Jiao X, Zhang S, Jiao J, Zhang T, Qu W, Muloye GM, Kong B, Zhang Q, Pedroza-Torres A, Campos-Parra AD, Millan-Catalan O, Loissell-Baltazar Fan L, Huang C, Li J, Gao T, Lin Z, Yao T. Long noncoding RNA urothelial carcinoma-associated 1 regulates radiosensitivity via targeting p21 in cervical cancer. Oncol Rep. 2018;39(3):1532–40.

Fan L, Huang C, Li J, Gao T, Lin Z, Yao T. Long noncoding RNA urothelial cancer associated 1 regulates radiosensitivity via the hexokinase 2/glycolytic pathway in cervical cancer. Int J Mol Med. 2018;42(4):2247–59.

Liu J, Liu X, Li R. LncRNA SNHG6 enhances the radiosensitivity and promotes the growth of cervical cancer cells by sponging miR-485-3p. Cancer Cell Int. 2020;20:84.

Choo YW, Kang M, Kim HY, Han J, Kang S, Lee JR, Jeong GJ, Kwon SP, Song SY, Go S, et al. Macrophage-Derived Nanovesicles Potentiate the Anticancer Efficacy of Immune Checkpoint Inhibitors. ACS Nano. 2018;12(9):8977–93.

Chu TY, Yang JT, Huang TH, Liu HW. Crosstalk with cancer-associated fibroblasts increases the growth and radiation survival of cervical cancer cells. Radiat Res. 2014;181(5):540–7.

Chung HC, Ros W, Delord JP, Perets R, Italiano A, Shapira-Frommer R, Manzuk L, Piha-Paul SA, Xu L, Zeilinger F, et al. Efficacy and Safety of Pembrolizumab in Previously Treated Advanced Cervical Cancer: Results From the Phase II KEYNOTE-158 Study. J Clin Oncol. 2019;37(7):1470–80.

Colombo N, Dubot C, Lorusso D, Caceres MV, Hasegawa K, Shapira-Frommer R, Tewari KS, Salman P, Hoyos Usta E, Yafeh E, et al. Pembrolizumab for Persistent, Recurrent, or Metastatic Cervical Cancer. N Engl J Med. 2021;385(20):1856–67.

Naumann RW, Hollebecque A, Meyer T, Devlin MJ, Okanin A, Kerger J, Lopez-Pica-Majo CM, Machiels JP, Delord JP, Evans TR, et al. Safety and Efficacy of Nivolumab Monotherapy in Recurrent or Metastatic Cervical, Vaginal, or Vulvar Carcinoma: Results From the Phase III CheckMate 358 Trial. J Clin Oncol. 2019;37(31):2825–34.

Kagabu M, NagaSawa T, Sato C, Fukagawa Y, Kawamura H, Tomabechi K, Takemoto S, Shoji T, Baba T. ImmunoTherapy for Uterine Cervical Cancer Using Checkpoint Inhibitors: Future Directions. Int J Mol Sci. 2020;21(7):2353.

Casper PF, McIlrath G, Gonzalez I, Wong N, Knudsen KE, Chen JJ, Markovina S, Schwarz J, Grigoryw PW, Wang X. Decreased local immune response and retained HPV gene expression during chemoradiotherapy are associated with treatment resistance and death from cervical cancer. Int J Cancer. 2020;146(7):2047–58.

Allouchi S, Malki A, Allouchi A, Gupta I, Vranic S, Al Moustafa AE. High-Risk HPV Oncoproteins and PD-1/PD-1 Interplay in Human Cervical Cancer: Recent Evidence and Future Directions. Front Oncol. 2020;10:914.

Zhang L, Zhao Y, Tu Q, Xue X, Zhuo X, Zhao KN. The Roles of Programmed Cell Death Ligand-1/Programmed Cell Death-1 (PD-L1/PD-1) in HPV-induced Cervical Cancer and Potential for their Use in Blockade Therapy. Curr Med Chem. 2021;28(5):893–909.

Danaei G, Latchman YE, Balint JP, Lee JH, Gabitzsch ES, Jones FR. An HPV-E6/E7 immunotherapy plus PD-1 checkpoint inhibition results in tumor regression and reduction in PD-L1 expression. Cancer Gene Ther. 2015;22(9):454–62.

Kim SS, Shen S, Myauchi S, Sanders PD, Franisk-Pietryga I, Mell L, Markovina S, Cohen EEW, Califano JA, Sharabi AB. Cells Improve Overall Survival in HPV-Associated Squamous Cell Carcinomas and Are Activated by Radiation and PD-1 Blockade. Clin Cancer Res. 2020;26(13):3345–59.

Xu S, Wang X, Yang Y, Li Y, Wu S. LSD1 silencing contributes to enhanced efficacy of anti-CD47/PD-L1 immunotherapy in cervical cancer. Cell Death Dis. 2021;12(4):282.

Wen Y, Zhang S, Yang J, Guo D. Identification of driver genes regulating immune cell infiltration in cervical cancer by multiple omics integrative Biomarker Pharmacochem. 2019;120:100546.

Bowden SJ, Bodinier B, Kalliala I, Zuber V, Doulgeraki T, Whithaker MD, Wilchers M, Cartwright R, Tsilidis KK, et al. Genetic variation in cervical preinvasive and invasive disease: a genome-wide association study. Lancet Oncol. 2021;22(4):548–57.

Franceschi C. Genomic characterisation of cervical cancer and human papillomavirus: new opportunities for precision medicine. Lancet Oncol. 2021;22(4):419–20.

Draghiuc I, Boerma A, Hoeoogboom BN, Nijman HW, Daemen T. A rationally designed combined treatment with an alphavirus-based cancer vaccine, sunitinib and low-dose tumor irradiation completely blocks tumor development. Oncomolecularology. 2015;4(10):e1029699.

Sun YY, Peng S, Han L, Qiu J, Song L, Tsai Y, Yang B, Roden RB, Trumble CL, Hung CF, et al. Local HPV Recombinant Vaccinia Boost Following Priming with an HPV DNA Vaccine Enhances Local HPV-Specific CD8+ T-cell-Mediated Tumor Control in the Genital Tract. Clin Cancer Res. 2016;22(5):657–69.

Peng S, Tan M, Li YD, Cheng MA, Farmer E, Ferrall L, Gaillard S, Roden RB, Hung CF, Wu TC. PD-1 blockade synergizes with intratumoral vaccination of a therapeutic HPV protein vaccine and elicits regression of tumor in a preclinical model. Cancer Immunol Immunother. 2021;70(4):1049–62.
170. Wendel Naumann R, Leath CA 3rd. Advances in immunotherapy for cervical cancer. Curr Opin Oncol. 2020;32(5):481–7.

171. Anderson AR, Weaver AM, Cummings PT, Quarta V. Tumor morphology and phenotypic evolution driven by selective pressure from the microenvironment. Cell. 2006;127(1):905–15.

172. Sottoriva A, Verhoeof JJ, Borovski T, McWeeny SK, Naumov L, Medema JP, Sloot PM, Vermeulen L. Cancer stem cell tumor model reveals invasive morphology and increased phenotypical heterogeneity. Cancer Res. 2010;70(1):46–56.

173. Meacham CE, Morrison SJ. Tumour heterogeneity and cancer cell plasticity. Nature. 2013;501(7467):328–37.

174. Gu W, Yeo E, McMillan N, Yu C. Silencing oncogene expression in cervical cancer stem-like cells inhibits their cell growth and self-renewal ability. Cancer Gene Ther. 2011;18(12):897–905.

175. Zhang J, Chen X, Bian L, Wang Y, Liu H. CD44+/CD24− Expressing Cervical Cancer Cells and Radioresistant Cervical Cancer Cells Exhibit Cancer Stem Cell Characteristics. Gynecol Obstet Invest. 2019;84(2):147–54.

176. Yang Y, Wang Y, Yin C, Li X. Clinical significance of the stem cell gene Oct-4 in cervical cancer. Tumour Biol. 2014;35(6):5339–45.

177. Tian Y, Ding W, Wang Y, Ji T, Sun S, Mo Q, Chen P, Fang Y, Liu J, Wang B, et al. Ubiquitin B in cervical cancer: critical for cancer stem-like cell characters. PLoS ONE. 2013;8(12):e84457.

178. Wang H, Cai HB, Chen LL, Zhao WJ, Li P, Wang ZQ, Li Z. STAT3 correlates with stem cell-related transcription factors in cervical cancer. J Huazhong Univ Sci Technolog Med Sci. 2015;36(5):891–7.

179. Liu W, Gao Q, Chen K, Xue X, Li M, Chen Q, Zhu G, Gao Y. Hiwi facilitates chemoresistance as a cancer stem cell marker in cervical cancer. Oncol Rep. 2014;32(5):1853–60.

180. Sato M, Kawana K, Fujimoto A, Yoshida M, Nakamura H, Nishida H, Inoue T, Taguchi A, Takahashi J, Adachi K, et al. Clinical significance of Gremlin 1 in cervical cancer and its effects on cancer stem cell maintenance. Oncol Rep. 2016;35(1):391–7.

181. Ortiz-Sanchez E, Santiago-Lopez L, Cruz-Dominguez VB, Toledo-Guzman ME, Hernandez-Cueto D, Muniz-Hernandez S, Garrido E, Cantu De Leon D, Garcia-Carranca A. Characterization of cervical cancer stem-like cells: phenotyping, stemness, and human papilloma virus co-receptor expression. Oncotarget. 2016;7(2):13943–54.

182. Cao HZ, Liu XF, Yang WT, Chen Q, Zheng PS. LGR5 promotes cancer stem cell traits and chemoresistance in cervical cancer. Cell Death Dis. 2017;8(9):e3039.

183. Leung CDN, Deng W, Ye TM, Ngn ANHS, Tsao SW, Cheung AN, Yin YN, Yuen DDK, Pang RTK, Yeung WSB. MicroRNA-125a-induced formation of CD133+ sub-population with cancer stem cell properties in cervical cancer. Carcinogenesis. 2020;41(11):1592–604.

184. Williams NL, Werner TL, Jarboe EA, Gaffney DK. Adenocarcinoma of the cervix: should we treat it differently? Curr Oncol Rep. 2015;17(4):17.

185. Stolniciu S, Barian I, Hoang L, Patel P, Teninte C, Pesci A, Aviel-Ronen S, Kiyokawa T, Alvarado-Cabrero I, Pike MC, et al. International Endocervical Adenocarcinoma Criteria and Classification (IECC): A New Pathogenetic Classification for Invasive Adenocarcinomas of the Endocervix. Am J Surg Pathol. 2018;42(2):214–26.

186. Lu Z, Chen J. Introduction of WHO classification of tumours of female reproductive organs, fourth edition. Zhonghua Bing Li Xue Za Zhi. 2014;43(10):649–50.

187. Park KJ. Cervical adenocarcinoma: integration of HPV status, pattern of invasion, morphological and molecular markers into classification. Histopathology. 2020;76(1):112–27.

188. Hodgson A, Olikov-Mislev E, Howitt BE, Nucci MR, Parra-Herran C. International Endocervical Adenocarcinoma Criteria and Classification (IECC): correlation with adverse clinicopathological features and patient outcome. J Clin Pathol. 2019;72(5):347–53.

189. Bulutay P, Haberal N, Ozen O, Erdem O, Zeren EH, Kulac I, et al. Reproducibility of Morphologic Parameters of the International Endocervical Adenocarcinoma Criteria and Classification System and Correlation With Clinicopathologic Parameters: A Multi-Institutional Study. Int J Gynecol Pathol. 2022;41(5):447–58.

190. Cree IA, White VA, Indave BI, Lokuhetty D. Revising the WHO classification of cervical tract histors. Histopathology. 2020;76(1):151–6.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.