Medical Oncology (2023) 40:149
https://doi.org/10.1007/s12032-023-01997-9

REVIEW ARTICLE

Molecular mechanisms augmenting resistance to current therapies in clinics among cervical cancer patients

Soumik Das[1] · Achsha Babu[1] · Tamma Medha[1] · Gnanasambandan Ramanathan[1] · Anirban Goutam Mukherjee[1] · Uddesh Ramesh Wanjari[1] · Reshma Murali[1] · Sandra Kannampuzha[1] · Abilash Valsala Gopalakrishnan[1] · Kaviyarasi Renu[2] · Debottam Sinha[3] · C. George Priya Doss[1]

Received: 23 January 2023 / Accepted: 10 March 2023 / Published online: 15 April 2023

Abstract

Cervical cancer (CC) is the fourth leading cause of cancer death (~324,000 deaths annually) among women internationally, with 85% of these deaths reported in developing regions, particularly sub-Saharan Africa and Southeast Asia. Human papillomavirus (HPV) is considered the major driver of CC, and with the availability of the prophylactic vaccine, HPV-associated CC is expected to be eliminated soon. However, female patients with advanced-stage cervical cancer demonstrated a high recurrence rate (50–70%) within two years of completing radiochemotherapy. Currently, 90% of failures in chemotherapy are during the invasion and metastasis of cancers related to drug resistance. Although molecular target therapies have shown promising results in the lab, they have had little success in patients due to the tumor heterogeneity fueling resistance to these therapies and bypass the targeted signaling pathway. The last two decades have seen the emergence of immunotherapy, especially immune checkpoint blockade (ICB) therapies, as an effective treatment against metastatic tumors. Unfortunately, only a small subgroup of patients (< 20%) have benefited from this approach, reflecting disease heterogeneity and manifestation with primary or acquired resistance over time. Thus, understanding the mechanisms driving drug resistance in CC could significantly improve the quality of medical care for cancer patients and steer them to accurate, individualized treatment. The rise of artificial intelligence and machine learning has also been a pivotal factor in cancer drug discovery. With the advancement in such technology, cervical cancer screening and diagnosis are expected to become easier. This review will systematically discuss the different tumor-intrinsic and extrinsic mechanisms CC cells to adapt to resist current treatments and scheme novel strategies to overcome cancer drug resistance.

Keywords Cervical cancer · Human papillomavirus · Metastasis · Recurrence · Drug resistance · Immunotherapy · Immune checkpoint blockade

Introduction

Cervical cancer (CC) is one of the common cancers that affect women around the globe. Although a highly preventable cancer, CC has a high morbidity rate (~300,000 deaths) among women globally and behaves epidemiologically like a low-infectious venereal illness [1]. A survey in 2018 predicted that ~2785 million women worldwide are at risk of getting CC, with an annual incident rate of ~569,847 diagnosed globally. Approximately four-fifths belong to poverty-stricken and lower-middle-income nations (LMICs) across Southeast Asia and sub-Saharan Africa. The persistent infection caused by Human Papillomavirus (HPV) high-risk subtype is considered the chief causative agent for the progression of CC. There has been a substantial decline in

Debottam Sinha and George Priya Doss have equally contributed to this work.

✉ C. George Priya Doss
georgepriyadoss@vit.ac.in

[1] School of Biosciences and Technology, Vellore Institute of Technology (VIT), Vellore, Tamil Nadu 632014, India

[2] Department of Biochemistry, Centre of Molecular Medicine and Diagnostics (COMManD), Saveetha Dental College & Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077, Tamil Nadu, India

[3] Faculty of Medicine, Frazer Institute, The University of Queensland, Brisbane, QLD, Australia

HPV transmission in nations implementing sufficient vaccination programs.

Australia is a good example of its vaccination program, where more than 70% of boys and girls aged 12–13 years nationwide participated. There was a reduction of 38% in the incidence of high-grade cervical dysplasia in girls in the age group under 18 years[2]. However, the World Health Organization estimates that developed countries with high vaccination rates will continue to die from HPV infections over the next 50 years due to existing infections and the long latent period before these cancers develop [3].

The standard of care treatment options includes surgical interventions, chemotherapy, and/or radiotherapy, alone or in combination. Advancement in the field has resulted in the development of molecular targeted therapy and immunotherapy (monoclonal antibody and cellular immunotherapy), either in clinical trials or FDA-approved. Interestingly, CC cells learn to adapt and resist these therapies in the clinic, causing poor pathological response alongside worse overall and relapse-free survival. Our review comprehensively discusses the current treatment regimens available to treat CC and the challenges faced in the clinic to design improved and effective therapeutic strategies for better clinical efficacy.

Pathophysiology of the disease and standard of care treatment

HPV infects the single-layered epithelial cells located at the cervical squamocolumnar junctions between the endocervix's columnar epithelium and the cervix's squamous epithelium between the endocervix's columnar epithelium and the cervix's squamous epithelium wherein only 3–5% of infection induces cellular transformation. The genome of the virus is composed of circular, double-stranded DNA, which encodes assembly (L1 and L2) proteins and viral replication proteins (E1 and E2/E4) alongside oncogenic proteins (E5, E6, and E7). These oncoproteins hijack the host cell's normal homeostasis, host cell's normal homeostasis, leading to cellular transformation and maintaining the malignant phenotype. Briefly, E6 and E7 disrupt the normal function of P53 and RB, which enables upregulation of survival and proliferation signaling cascade causing abnormal cell growth. The treatment of CC in its early stages involves surgery significantly. A type III radical hysterectomy with bilateral pelvic lymphadenectomy is done in the usual surgical technique [4]. Radical hysterectomy, total hysterectomy, trachelectomy, loop electrosurgical excision procedure (LEEP), cervical conization, and cryosurgery remain the mainstay to treat CC. The use of radiotherapy, especially brachytherapy (internal RT), intensity-modulated radiotherapy (IMRT), and external beam radiation therapy (EBRT), emphasizes the therapeutic advantages of adaptive radiotherapy (ART) methods for treating CC [5].

Chemotherapy is an essential component of the standard CC treatment schedule administered in an adjuvant setting, combined with radiotherapy post-surgery upon poor prognostic tumor features dictating a high risk of recurrent disease. Platinum-based drugs like cisplatin combined with other non-Pt-based drugs are used to treat CC. Other promising anti-cancer agents include nano-sized phytochemicals (NPCs) since they only need to be used in minimal amounts, reducing the overall treatment cost. Enhanced drug selectivity, increased absorption rates, less drug degradation, and decreased systemic toxicity are all excellent benefits of nano-scale drug delivery devices [6]. In patients with locally advanced CC, dose-dense Paclitaxel in neo-adjuvant chemotherapy combinations is a viable treatment strategy. Investigating innovative biological treatments and in vitro combinations that use Paclitaxel is necessary [7]. Brachytherapy, a crucial therapeutic approach to delivering enough dosages to the peripheral and central regions of cervical carcinomas, can increase the rates of remission, recurrence, and survival for all CC types.

Due to poor water solubility, low oral bioavailability, and the need for high doses, phytochemicals as therapeutic drugs are constrained. However, they can inhibit cancer development by interfering with nearly every stage of carcinogenesis. Notably, a wide range of side effects, such as toxicity, hair loss, anemia, neurotoxicity, non-targeted tissue damage, multidrug resistance (MDR), neutropenia, and nausea, are frequently experienced by patients taking chemotherapy or radiotherapy [6]. As such, the concept of molecular targeted therapy as anticancer agents was initiated over the last two decades with the notion of causing minimum toxicity as non-neoplastic host cells have limited distributions of cell surface receptors and intracellular targets. The rationale behind molecular engineering therapies was specifically targeting validated molecular receptors regulating cancer-signaling pathways. However, the targets also exist on or within normal cells; even receptors on nontarget tissues are cross-reactive. These therapies have revolutionized cancer treatment against multiple aggressive cancers with poor prognoses. Still, they are now associated with long-term survival, such as chronic myeloid leukemia, breast cancer melanoma, colorectal, lung, and even renal cell carcinoma.

The magic of molecularly targeted therapies

Small molecule inhibitors (SMIs) are novel targeted therapy drugs that are easier to design structurally to satisfy clinical needs due to their convenience and affordability. Receptor tyrosine kinases are well known to be implicated in tumorigenesis and progression and have emerged as major targets

for SMIs. Their degradation has been the primary focus for the development of molecular therapies as TK inhibitors (TKIs), which target and block these enzymes, are crucial in the fight against different forms of cancer [8]. Apatinib, a brand-new oral tyrosine kinase inhibitor targeting the VEGFR2 signaling pathway, has demonstrated promising therapeutic results in various malignant cancers. Apatinib has anti-cancer effects in cancer types, such as breast, gastric, ovarian, non-small-cell, non-small-cell anti-cancer effects in various cancer types, including breast cancer, gastric cancer, ovarian cancer, non-small-cell lung cancer, gastric, and hepatocellular carcinoma.

In vitro and animal models, apatinib greatly enhanced the paclitaxel sensitivity of cervical cancer cells, according to a recent study [9, 10]. It also reduced microvascular tumor density and blocked the development of new blood vessels in tumor tissue. A good example is patients with HER2-mutant CC who had received much prior treatment demonstrated activity with neratinib monotherapy but no new safety signals [11].

The VEGF family plays a critical role in tumor invasion and metastasis among CC patients, and stabilization of HIF-1 alpha alongside the suppression of p53 by HPV appears to be directly related to the predominant role of angiogenesis in CC and may elevate VEGF. Different VEGF inhibitors, such as bevacizumab, pazopanib, lapatinib, brivanib, and sunitinib, have been studied and tested recently and have significantly reduced cervical cancer development. In a recent Gynecologic Oncology Group (GOG) phase III trial, the inactivation activity of bevacizumab has aided in reducing the progression of CC [12]. Pazopanib and lapatinib focus on the c-Kit or EGFR and HER2/neu and platelet-derived growth factor receptor VEGFR. High microvascular density and EGFR and HER2/neu overexpression are correlated with survival in cervical cancer [13]. EGFR is highly overexpressed in CC (70–90% of cases), and EGFR tyrosine kinase inhibitor, Canertinib, has been shown to irreversibly inhibit all four members of the EGFR family. In cervical cancer, it also slows the development of cancer cells and triggers apoptosis [14]. The novel 6-benzoyl benzimidazole derivatives as an EGFR inhibitor were tested as cytotoxic agents against CC cells by Eman et al. in 2020 [15].

Similarly, erlotinib and bevacizumab have been used in targeted cervical squamous cell carcinoma therapies, but their efficiency in inducing anti-tumor activity is limited. It could be due to the complexity of cell signaling, clonal heterogeneity, and intra-tumor genetic heterogeneity. The first known mTOR inhibitor was rapamycin, found during an anti-microbial compound search in 1975 [16]. Rapamycin's low solubility in water and its chemical stability prevented the clinical outcome as an anti-cancer drug. As a result, numerous rapamycin analogs (rapalogs), including temsirolimus and everolimus, are now being tested in clinical studies for cancer treatment [17]. These drugs have enhanced pharmacokinetic features and reduced immunosuppressive effects. Studies conducted in vitro have shown that mTOR inhibitors limit the development of CSCC cells. In CSCC cell lines, mTOR inhibitors have been shown to have various effects [18]. Depending on the kind of cell, curcumin prevents the multiplication of cancer cells by halting them at various stages of the cell cycle. It is important to research mTOR inhibitors as CC therapies, particularly in combination with radiation. If the selectivity of diet-derived mTOR inhibitors for cancer cell lines is established, they may be effective treatments for CC. PI3K inhibitor (BYL-719/LY294002) has been shown to overcome paclitaxel-mediated resistance alongside suppression of tumor migration and invasion in preclinical CC setting [19]. The molecular targeted therapy for CC is tabulated in Table 1.

The therapeutic targeting of the remnant DNA damage response (DDR) is an intriguing method of chemo radiosensitization for CC via this HPV-mediated partial inactivation of the DDR [30]. DNA repair capacity appears to limit treatment responses, and combined chemoradiotherapy is the norm for advanced CC treatment. Therefore, therapeutic regulation of the DDR is a primary alluring method to increase the effectiveness of CC treatment. Because the DDR is partially damaged in HPV-mediated CC, cancer cells may depend more on any remaining DDR signaling axis [31]. In tumors linked to abnormalities in DNA repair, poly(adenosine diphosphate [ADP]-ribose polymerase (PARP) inhibitors (PARPi) have become a potential class of chemotherapy drugs [32]. PARPi targets PARP, such as olaparib, which has become popular in treating CC [33]. Cells representing cervical squamous cell carcinoma and adenocarcinoma may develop less rapidly when treated with PARPi.

Additionally, adding PARPi to cisplatin made CC cells more susceptible to the cytotoxicity that cisplatin causes [34]. Only one phase 1 (NCT01281852) study has evaluated veliparib in combination with Paclitaxel and cisplatin in persistent or recurrent CC. As part of a phase 2 study, PARPi and MaRuC (NCT02795272) are being investigated as maintenance therapy for advanced CC. Niraparib combined with pelvic radiation followed by induction chemotherapy is being studied in the phase 1/2 trial NIVIX (NCT03644342) for treating metastatic stage IV invasive CC [35]. In a phase 2 research called Clovis-001 (NCT03476798), women with recurrent cervical or endometrial cancer are studied with bevacizumab and rucaparib. The phase 2 KEYNOTE-158 study (NCT02628067) has already demonstrated the promise of immunotherapy for CC. Therefore, PARPi-based immunotherapy may be useful for CC [36]. However, in clinical practice, targeted therapy fails to work in the long run due to tumors not responding to the drugs or the person who

Table 1 Case studies on molecular target therapy for CC are undergoing clinical trials

Targets	Drugs	Concentration administered	Trial Phase	Stage	Population	References
VEGF	Bevacizumab	10 mg/kg intravenously every 2 weeks for three cycles	Phase II	Patients with bulky tumors (Stage IB-IIIB)	60 patients	[20]
		15 mg/kg intravenously every 21 days	Phase II	Patients had recurrent CC	46 patients	[21]
	Pazopanib and lapatinib	Pazopanib at 800 mg once per day, lapatinib at 1500 mg once per day	Phase II	Stage IVB persistent/recurrent cervical carcinoma	152 patients: pazopanib ($n=74$) or lapatinib ($n=78$)	[13]
	7-Difluoromethyl-5,4'-dimethoxygenistein (DFMG)	50 µM DFMG	–	Siha cells	Cell line study of CC	[22]
Anti-VEGF tyrosine kinase inhibitors	Sorafenib	Sorafenib at 200 mg twice daily and four at 400 mg twice daily	Phase 1	stage IB to IIIB CC	13 patients	[23]
COX-2	Celecoxib	Celecoxib-400 mg po twice daily	Phase II	carcinoma of the uterine cervix; FIGO stage IIB-IVA or FIGO Stage IB-IIA disease	78 Patients	[24]
		400 mg twice daily together with concurrent cisplatin	Phase II study	Advanced cervix cancer; FIGO Stage IIB-IVA or patients with FIGO Stage IB through IIA	84 patients	[25]
	Rofecoxib	25 mg for six months	Grade II-III A phase II trial	Cervical intraepithelial neoplasia (CIN) grade II and III	16 Patients	[26]
mTOR	Erlotinib	a dose of 150 mg/day one week	Phase II	Stage IIB to IIIB epidermoid CC	36 patients	[27]
EGFR signaling pathway	Gefitinib	500 mg/day gefitinib	Phase II	squamous-cell carcinoma or adenocarcinoma	30 patients	[28]
PARP inhibitors	Rucaparib	Rucaparib was given at 600 mg BID twice daily for each 21-day cycle	Phase II	Recurrent cervical or endometrial cancer	33 patients	[29]

VEGF vascular endothelial growth factor, *DFMG* difluoromethyl-5,4'-dimethoxygenistein, *COX-2* Cyclooxygenase-2, *FIGO* international Federation of Gynecology and Obstetrics, *EGFR* epidermal growth factor receptor, *PARP* poly-ADP ribose polymerase, *mTOR* mammalian target of rapamycin

initially responded having eventually developed acquired resistance to the drugs [37].

Therapeutic vaccine

The global incidence of cancer is on the rise, and there is an urgent need to improve therapy. Therapeutic vaccines might play a key role in controlling CC [38]. Therapeutic vaccinations strengthen the immune system to eradicate already-developed cancer. A therapeutic vaccine is anticipated as an alternative to an antibody-based medication [39]. The main goal of therapeutic vaccines is to trigger an immune response against tumor antigens, which results in tumor regression [40]. Therapeutic vaccinations come in a variety of forms that are used to stimulate the immune system. Table 2 lists the vaccines studied around the world against CC.

Table 2 List of vaccine studies carried out worldwide

Study reference	Vaccine approaches	Description	ClinicalTrials.gov identifier	Treatment	Indications
[41]	Tumor cell-based vaccines	Autologous—Tumor cells are taken out of the same patient undergoing treatment	NCT00052156	Canvaxin with Bacille Calmette-Guerin	Melanoma
[42]	Tumor cell-based vaccines	Allogenic—Any individual other than the one being treated is used for getting tumor cells	NCT01696877	Gvax	Pancreaticcancer
[43]			NCT00676507	Belagenpumatucel-L	Non-small cell lung cancer
[44]	Dendritic cell vaccine	sDC serves as antigen-presenting cells to induce an antigen-specific T-cell response	NCT01431391	Sipuleucel-T	Prostate cancer
[45]	DNA vaccine	It generates an immune response using genetically modified DNA	Phase III (Current)	ZyCoV-D	COVID-19
[46]			NCT03439085	MEDI0457	Head and Neck Cancer
[47]	Peptide Vaccine	It uses short peptide fragments to create highly targeted immune responses	NCT02454634	IDH1	Glioma
[48]			NCT04780035	EpiVacCorona	COVID-19
[49]	Live vector-based vaccine	Viral Vector- Delivers genetic material coding for a desired antigen into the recipient's host cells using a viral vector	NCT00116155	CG7870	Prostate cancer
			NCT03799744	VCN-01 with Durvalumab	Head and neck cancer
[50]	Live vector-based vaccine	Bacterial Vector—Uses live bacteria as a vector to deliver heterologous antigens	NCT02853604	ADXS11-001	CC

Tumor cell-based vaccines

Tumor cell-based vaccines were the first therapeutic vaccines designed to treat cancer. The tumor cells are removed from the body and treated with radiation and chemicals, which along with adjuvants, are injected back into the patients to boost their immune response. The benefit of the tumor cell vaccine is that before administering the vaccination, one does not need to be aware of the precise antigen, e.g., in the melanoma vaccine, canvaxin uses autologous tumor cells and BCG as an adjuvant. Thus, the immune response is enhanced by the granulocyte–macrophage colony-stimulating factor (GM-CSF) [51].

Live viral vector vaccine

A live viral vector vaccine is a viral vector vaccine that offers resistance by using a modified form of a different virus as a vector [52]. This type of vaccine is used against the vaccinia virus, vesicular stomatitis viruses, adenoviruses, alphaviruses, and adeno-associated viruses. HVP, the predominant virus of CC, is targeted by Vvax001, an alphavirus-based therapeutic cancer vaccine under phase I of clinical trials [53].

Live bacterial vector vaccine

A live bacterial vector vaccine, a perfect vaccine, should be capable of stimulating both the infected host's innate and adaptive immune systems. An innovative and efficient option for creating novel vaccines is using live bacteria as a vehicle to deliver heterologous antigens. A phase I clinical trial using recombinant L. monocytogenes Lm-LLO-E7 (ADXS11-001) was conducted on late stage, metastatic CC patients who had previously failed chemotherapy, radiation, or surgery. Four of the thirteen individuals evaluated in a trial had their tumor load reduced. A randomized, single, placebo-controlled phase II research in a cohort of 120 individuals with CIN2/3 and a multicentre phase II clinical trial in 67 patients with persistent or recurrent CC are now evaluating ADXS11-001 (NCT01116245) [54].

Peptide vaccines

Peptide vaccines are non-auto immunogenic antigens from cancer proteins that can stimulate immunity and eliminate CC [55]. E6/E7 peptide vaccines with pegylated INF-α as adjuvant against CC have reached clinical trials. Some ongoing peptide-based HPV therapeutic vaccines are being investigated, like HPV-16 E6/E7 derived epitopes against cervical intraepithelial neoplasia (NCT02065973), four HPV-16 peptides with candin to determine tolerance, dosage, and immunogenicity in women with high-grade squamous intraepithelial lesion (HSIL) lesions (NCT01653249) [56].

Dendritic cell vaccine

To regulate pathogens via the innate immune system and to realize immunological memory, DCs stimulate NK cells, which, in turn, stimulates adaptive immunity [57]. In response to the antigen presented, DCs and T cells develop immunological synapses that promote T-cell activation [58]. This equilibrium could also be manipulated through DC vaccination therapy for therapeutic purposes. In an investigation, 15 patients with stage IV CC received autologous monocyte-derived DCs pulsed with recombinant HPV16 E7 or HPV18 E7 oncoprotein. There were no adverse reactions or toxicity associated with the vaccination. It was observed that in some patients of late-stage CC, T-cell responses could be triggered by dendritic cells pulsed with HPV E7 protein [59].

DNA vaccine

Simple DNA rings that contain an antigen-coding gene and a promoter/terminator that causes the gene to express in mammalian cells make up the DNA vaccines [60]. A study found that GX-188E immunization caused individuals with cervical precancer to experience cervical lesion reduction and HPV E6 and E7 specific T-cell responses. With the GX-188E therapeutic vaccination combined with pembrolizumab, patients with recurring or advanced CC had safe treatment. In this interim analysis, the combination therapy demonstrated tentative anti-tumor efficacy, offering a novel therapeutic approach to this patient population [61]. The therapeutic vaccines used against CC are illustrated in Fig. 1.

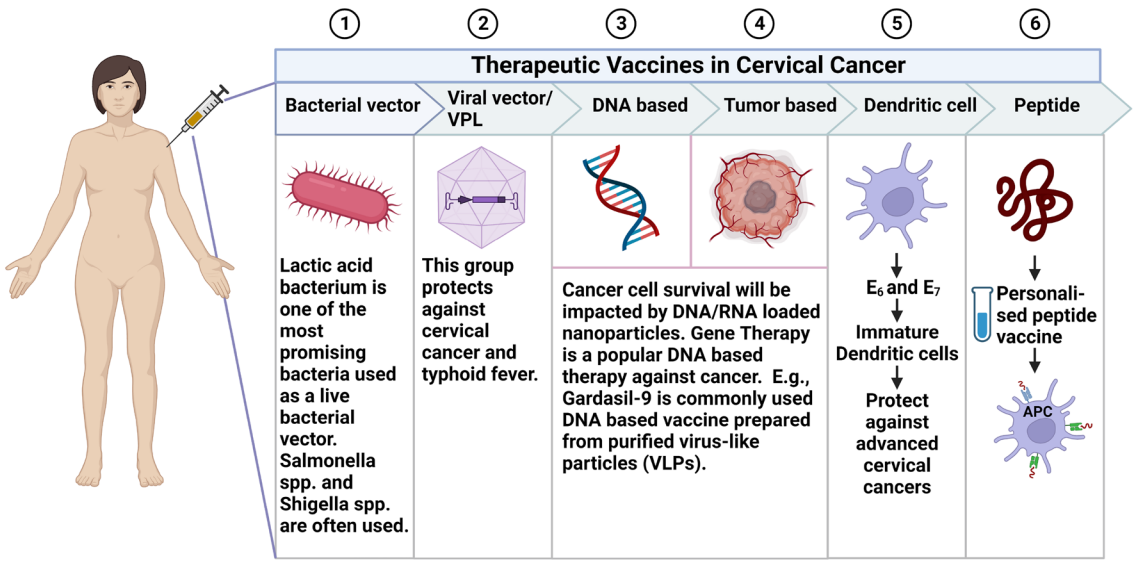

Fig. 1 Summary of the main types of therapeutic vaccines used against CC

The rise of immunotherapies

Cancer immunotherapy, also called immuno-oncology, is a cancer treatment that can enhance or alter how the immune system works to prevent, control, and eliminate cancer [62]. Immunotherapy boosts the host immune system to fight against the cancer cells, which eradicate malignancy by selectively recognizing the virus-infected cells. Worldwide, CC affects 7.9% of all females and is women's most prevalent cancer type. HPV infections with the potential to cause cancer account for 95% of cases of CC. The most carcinogenic HPV is HPV16, which has the highest prevalence of CC and grade 3 intraepithelial neoplasia [63]. Vaccination, either as a prophylactic or therapeutic approach, was the first step towards developing an immunotherapeutic tactic initiated to boost immunity and eliminate CC. Prophylactic vaccines, such as Gardasil 9, were developed and refined to protect against nine types of high-risk HPV strains, and multiple countries around the globe have adopted this vaccination program. Similarly, strategies to develop a therapeutic vaccine, an alternative to an antibody-based medication [39], are currently being optimized to trigger an immune response against tumor antigens, which results in tumor regression [40]. These vaccines take advantage of constitutively produced tumor-specific antigens E6 and E7, which activate and proliferate T lymphocytes that selectively target and destroy cancer cells [64].

Immunomodulatory drugs like imiquimod and gemcitabine (GEM) are currently used to treat CC. Imiquimod and its analogs are being investigated for their precise mechanisms of action. However, imiquimod is known to promote the release of interferon-alpha (IFN-alpha), IL-6, and TNF-alpha and activates immune cells through TLR-7 [65, 66]. Numerous cancers have responded well to immunomodulatory therapy, including melanoma, renal cell carcinoma, and non-small cell lung cancer [67]. Checkpoint inhibitors block the immune system's inhibitory receptors by activating the immune cells to fight against tumors [68]. *PD-1 and PD-L1* immune regulatory axes are promising targets for CC treatment. A humanized monoclonal IgG4 kappa isotype antibody targets PD-1 [69]. In a recurrent PD-L1 positive CC clinical study, when the patients were treated with 10 mg/kg of pembrolizumab every two weeks up to two years, the overall response rate (ORR) was seen to be 17% [70]. Similarly, in a recurrent, metastatic, HPV + CC clinical study, when the patients were treated with 240 mg of Nivolumab every two weeks up to 2 years, ORR was 26.3% [71]. In 2020, in a recurrent and metastatic CC clinical study when the patients were treated with only balstilimab of 3 mg/kg every two weeks up to 2 years showed an ORR of 14%, while when balstilimab (3 mg/kg) was given along with zalifrelimab (1 mg/kg), the ORR increases to 22% [72]. Recruitment is now available for the phase I study trial with the International Federation of Gynecology and Obstetrics (FIGO) stage IB to IV patients and the phase II study of pembrolizumab combined with chemo, radiation, and brachytherapy [73]. For the treatment of CC, additional checkpoint inhibitors, such as Atezolizumad, Durvalumab, and Nivolumab, are

Table 3 List of immunotherapy studies carried out worldwide

Study reference	Immunotherapy approaches	Description	ClinicalTrials.gov identifier	Treatment	Indications
[74]	Immune checkpoint inhibitors	These proteins are barriers, preventing the immune system from attacking cancer cells	NCT02311361	Durvalumab with tremelimumab	Pancreatic cancer
			NCT02303990	Pembrolizumab	Metastatic cancer
			NCT01375842	Atezolizumab	Hematologic Malignancies
[38]			NCT02628067	Pembrolizumab	CC
[75]	Immunomodulatory Therapy	A method of treating cancer by enhancing or triggering immune system defenses against the disease	NCT04273529	Thalidomide	COVID-19
[76]			NCT01750983	Lenalidomide with ipilimumab	Advanced Cancers
			NCT01553149	Lenalidomide	Astrocytoma Glioma
[77]	Adaptive immunotherapy	Treatment that eradicates cancer using immune system cells	NCT02541370	CART-cell	Malignancies

CART cell chimeric antigen receptor T-cell, *CC* cervical cancer

also being investigated. Immunotherapy studies carried out worldwide in CC are documented in Table 3.

Adaptive T-cell therapy is recommended as a potentially curative treatment for people with metastatic CC. In adoptive T-cell treatment, tumor samples are cultured ex vivo, and tumor-infiltrating lymphocytes (TILs) are amplified [78]. These T cells are injected into autologous tumor-bearing patients after lymphodepletion treatment. In patients with metastatic solid tumor malignancies, a phase I research investigated adoptive CD4 + T-cell treatment employing retroviral transduction of a T-cell receptor that recognized the melanoma-associated antigen-A3. In the trial, there were two CC patients; one after 29 months of treatment, i.e., no signs of cancer after the treatment [79]. Patients with HPV-associated malignancies are enrolled in a Phase I trial using T-cell receptor treatment targeting the HPV-16 E7 oncoprotein alone or in conjunction with the PD-1 inhibitor Pembrolizumab.

Mechanism of resistance

CC patients with advanced or recurrent disease demonstrate poor prognosis as the disease engineers multiple resistance mechanisms, including reduced uptake of drugs, adaptive somatic mutations, and heterogenous genetic landscape, cancer stem cell replenishing, rewiring of the signaling cascade, hostile tumor microenvironment and inadequate trafficking of drugs to the tumor site. Intrinsic genomic instability facilitates an escape route for CC cells when challenged with cytotoxic or targeted therapies [80]. Resistance to cancer immunotherapies is dictated by an interplay between tumor-cell-intrinsic and tumor-cell-extrinsic factors, which ultimately results in immunosuppression and evasion due to hijacking the efficacy of T cells to recognize or present tumor antigens. The intrinsic tumor factors comprise the expression or repression of certain genes and pathways in tumor cells, which regulate immune cell infiltration within the tumor stroma. The extrinsic factors include T-cell absence, downregulation of tumor antigen presentation, inhibitory immune checkpoints, and immunosuppressive cells [81]. Understanding these mechanisms is highly warranted for designing novel therapeutic strategies. Hence we have elaborated on these mechanisms shedding light on the current need for cancer therapeutics in the clinic.

Somatic and germline mutations

Genetic mutation is one of the prime reasons for cancer development, which alters the gene's normal function. Germline mutations are changes to DNA in the gametes that are inherited during conception, while somatic mutations occur in somatic cells after conception. Somatic and germline mutations exist in primary mismatch repair genes such as *MSH2*, *MSH6*, *MLH1*, and *PMS2* which have microsatellite instability. Genetic characterization of tumors shows mutations in *BRCA2* and *MLH1* genes as germline mutations in CC. Most cervical tumor mutational landscapes were found in *PIK3CA*, *KRAS*, *FBXW7*, *ALK*, and *EGFR*, with 48% deleterious cancer mutations [82]. *SKT11* is a tumor suppressor gene. Germline mutation of this gene increases the risk of CC by 10% [83]. A study by Wingo et al. showed a somatic mutation of 20% in CC of the tumor suppressor gene *LKB1* [84]. Next-generation sequencing (NGS), including whole-genome sequencing (WGS) and whole-exome sequencing (WES), is rapidly detecting and characterizing the genomic DNA. They are likely to have a great impact on the study of mutagenesis. This mutagenic analysis will greatly help the clinician diagnose CC [85]. In a separate study, NGS identified *HLA-B*, *PIK3CA*, *KMT2D*, *FAT1*, *mTOR*, and *ZFHX3* as frequently mutated genes in CC. It also found somatic mutations in *MDC1*, *ANKRD11*, *APC*, *BCORL1*, *BRCA1*, *CHD1*, *KRAS*, *FBXW7*, and *TP53,* while germline mutations in ATM, and RAD51B. PI3K/Akt/mTOR signaling pathway is activated in CC by mutation of *PIK3CA* and *mTOR* [86].

Genomic instability and aneuploidy

Genome instability causes genetic mutations where normal cells can degenerate into malignant cells. The tumor suppressor genes lose their function and activate the oncogenes. Due to this, cancer cells demonstrate substantial heterogeneity as the disease advances. Genome alterations brought on by chromosome segregation, DNA replication, and telomere maintenance are crucial for tumorigenesis [87]. Individuals with shorter cell cycles and/or advantages in evading intracellular and immune regulatory systems due to genomic instability are more likely to grow and be chosen to undergo malignant transformation [88]. In the presence of chemo and molecular therapies, the tumor cells still survive. This is brought on by the epithelial-mesenchymal transition (EMT), cell cycle dysregulation, cancer stem cells (CSCs) resilience, and repair response to radiation-induced DNA damage [89]. Exome sequencing in clinical practices identifies rare diagnoses that were earlier expensive, time-consuming, and risky during invasive procedures. They provide more genetic information, which is beneficial for identifying genetic alterations brought on by disease [90].

Signaling pathway network rewiring

Like any other cancer, CCs are detected in the late stage. For effective treatment, an urgent need is to develop an early diagnosis. In CC, molecular pathways have emerged as promising therapeutic targets. ERK/MAPK pathway regulates cell differentiation, proliferation, angiogenesis,

and survival. The epidermal growth factor (EGF) binding to its receptor, EGFR, leads to GRB2/SHC/SOS activation, which activates the RAF/MEK/ERK pathway triggering a series of phosphorylation events. The critical molecular signal regulators are RAS and RAF. MEK1 and MEK2 regulate the intermediate signaling, which phosphorylates and activates ERK1 and ERK2. ERK1/2 acts on substrates in the cytoplasm and nucleus to regulate cellular activity. In CC, RAS is commonly activated and responsible for tumor metastasis. The development of recurrent CC is seen when RAS and Myc are mutated. Even overexpression of EGFR is seen in CC. Targeting kinases like RAF and MEK can benefit CC therapy [91].

PI3K of PI3K/Akt pathway downregulates RAS signaling. Activated PI3K converts Ptd(4,5)P_2 to Ptd(3,4,5)P_3. Akt and PDK1 bind to Ptd(3,4,5)P_3 and are hired at the PI3K activation sites. The proteins recruited help PFK1 to phosphorylate Akt. On phosphorylation, the catalytic activity of Akt is stimulated, which phosphorylates other proteins and affects the proliferation of cells, cell cycle entry, and anti-apoptosis. Degradation of Ptd(3,4,5)P_3 by SHIP and PTEN terminates PI3K. In CC, deletion of PTEN and upregulation of PI3K are often seen enhancing the Ptd(3,4,5)P_3 synthesis. Inhibition of PI3K or Akt and even downstream targets like mTOR will provide maximum inhibition. HCCR oncogene regulates PI3K/Akt signaling in CC. Activating the PI3K/Akt signaling pathway results in tumor growth and survival [91].

The JAK-STAT3 pathway is associated with proliferation, invasion, survival, inflammation, and immunity. On activating the JAK-STAT3 pathway, hematopoietic cells proliferate, whereas epithelial cells form cell adhesions. The membrane-bound cytokine receptor gets activated by an interferon/interleukin. They recruit intracellular tyrosine kinases, JAK, upon activation, to the cytoplasmic domains. The JAK phosphorylates tyrosine residues of the receptor. STAT proteins carry the sh2 domain and are thus able to bind to the phosphorylated tyrosine residue of the receptor. STATs get phosphorylated, dimerized, and enter the nucleus, where they bind to specific promotor motifs of the DNA, CREs. DNA-bound STAT activates the transcription of many target genes. In CC, cytokine receptors' overexpression and even persistent activation or overexpression of STATs leads to poor overall survival. STAT inhibition offers hope for developing novel cancer therapeutic targets [92]. The involvement of cellular signaling pathways in CC development or progression is illustrated in Fig. 2. Analyzing these protein targets in body fluids, proteomics will help identify and monitor biomarkers to help create personalized drugs by understanding the protein interaction and its relation to the disease pathway [93].

Hypoxia and low nutrients

A well-known feature of solid tumors is hypoxia, an established therapeutic target. Tumor hypoxia results from inadequate oxygen supply to the tumors. This phenomenon is associated with tumor progression and resistance to therapy. The mitochondrial oxygen consumption and ATP generation are reduced in hypoxia. This results in proteome change [93]. Glucose is the primary source of energy for hypoxic tumors, which utilizes glycolysis to secrete lactate. The oxygenated tumor cells absorb lactate to provide for their energy requirements. IL-6 secreted by hypoxic tumors triggers both STAT3 and MAPK signaling pathways, which enhances metastasis. It is observed that PI3K/AKT activation increases VEGF secretion in both HIF-1-dependent and independent manner. Hypoxia is the critical factor for ROS accumulation in cancer cells [94]. A study by Palan et al. revealed that the β-carotene level was lower in CC patients than normal. Low carotenoids lead to neoplasia [95]. Many case studies indicated that low concentrations of vitamin C (ascorbic acid) and vitamin E (α-tocopherol) are responsible for cervix inflammation. A high intake of such deficiencies can improve the condition. Low folate intake leads to CC. In the early stage, increasing folate intake reduces the risk of cancer. Likewise, low serum ferritin, low dietary iron, high fat intake, and cruciferous vegetables are responsible for the enhanced risk of CC. Thus, ascorbate, carotenoids, and tocopherols are antioxidants that protect DNA, protein, carbohydrate, and lipids from ROS activity [96].

Hostile tumor microenvironment

The tumor cells draw an extracellular matrix, leukocytes, cancer-associated fibroblasts, endothelial cells, and pericytes to the primary tumor site. It creates the tumor microenvironment, which aids in cancer development and metastasis. TGF-β secreted by tumor cells leads to developing fibroblasts linked to cancer (CAFs). CAFs encourage angiogenesis, cell–cell communication, and cell proliferation. Stimuli from the tumor microenvironment lead CAFs to undergo EMT. CAFs stimulate the surrounding cells to become malignant. CAFs secrete SDF-1, which recruits EPCs to tumor mass and stimulates angiogenesis. As a result, CAFs are crucial for therapeutic purposes and offer a favorable tumor microenvironment. To promote immune evasion and tumor growth, chemokines and cytokines are introduced into the tumor microenvironment and activate various inflammatory cells. The tumor cells produce VEGF, M-CSF, and MCP-1, which recruit macrophages into the tumor microenvironment. They produce signaling (chemokines) molecules that work together and activate integrin α4β1, entering the tumor microenvironment and developing primary tumors. Together with CAFs, CXCL12 creates an inflammatory

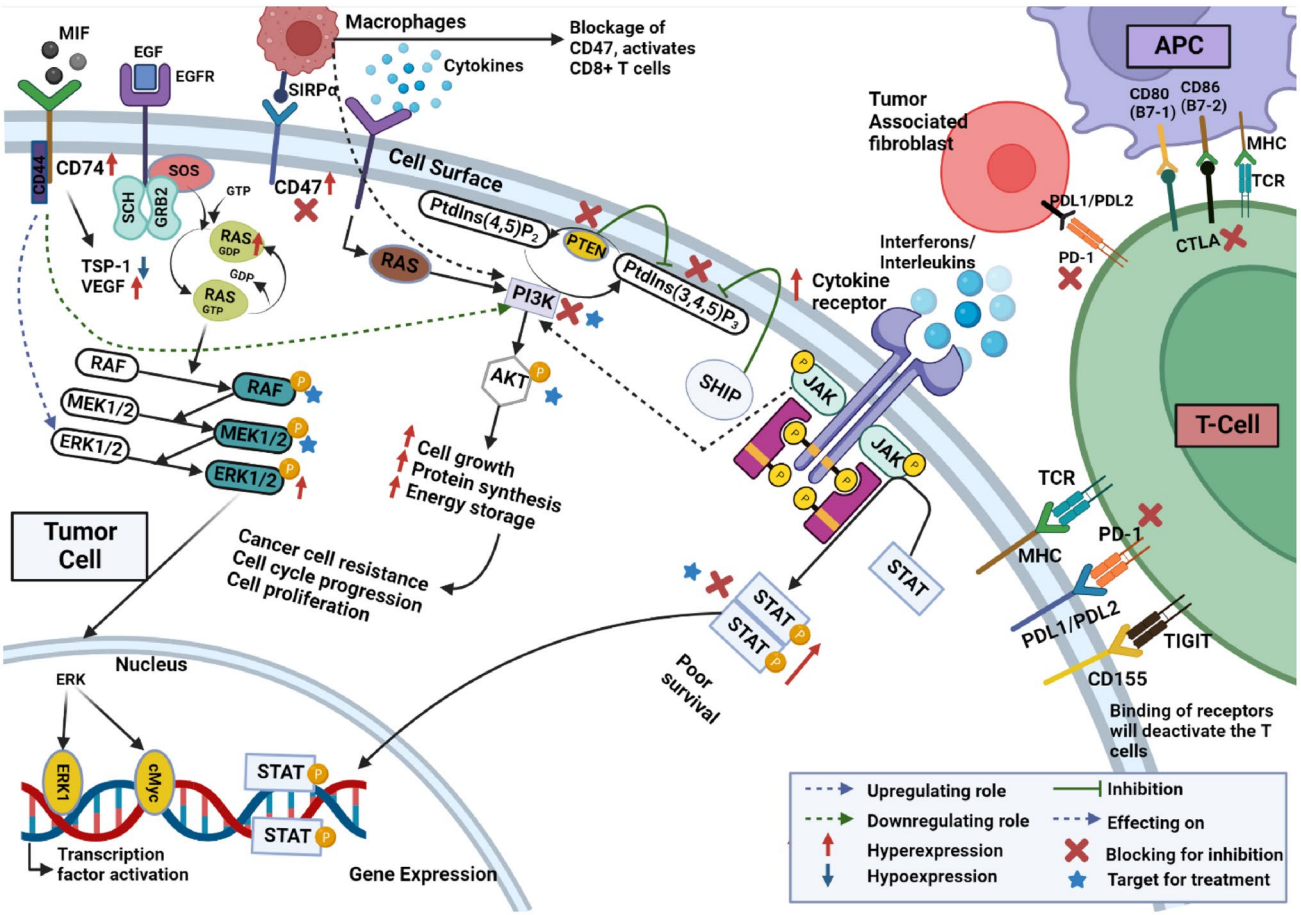

Fig. 2 The involvement of cellular signaling pathways to CC development or progression. It has been shown that several pathways play important roles in the development or spread of CC. HPV may improve or impair signaling networks' typical operations. In turn, HPV E6 and E7 could activate PI3K/AKT by raising the expression of both PI3K and AKT. By lowering the expression of p53, these HPV oncoproteins prevent the occurrence of apoptotic signaling pathways. Additionally, by increasing the number of EGFRs on the surface of cells infected with HPV, E5 stimulates the PI3K/AKT and MAPK/ERK signaling pathways. It also shows how the loss of antigen presentation will modulate the expression of PDL-1, CD155, CD74, and CD47

environment that guards against immune destruction in tumor cells [97, 98]. miRNAs in exosomes play an essential role in the tumor microenvironment, bridging cancer and stromal cells, e.g., miR-21, and miR-29a bind to human TLR8 and trigger a TLR-mediated pro-metastatic inflammatory response leading to tumor metastasis [99]. Tumors contain all amino acids except glutamine. CAFs undergoing autophagy supply a high glutamine level to the tumor microenvironment. As a result, mitochondrial activity in cancer cells increases. Inducing HiF1 and activating NF-B, accumulated ROS from cancer cells is transported to nearby fibroblasts, causing oxidative stress that promotes autophagy. It leads to DNA damage and cancer development [100]. The "angiogenic switch" is turned on in the tumor microenvironment, which sustains tumor angiogenesis, e.g., TAMs and Tie2 expressing monocytes (TEMs) promote tumor-associated angiogenesis. Stromal cells mediate tumor metastasis. The tumor microenvironment contributes to tumor metastasis. The tumor microenvironment provides a safe zone for cancerous cells. Targeting the microenvironment will be of great therapeutic potential [99].

Tumor antigen presentation

Clinically useful CD8 + T-cell responses focus mostly on neoantigens, antigens produced from tumor-specific mutations that accumulate in malignancy [101]. Class I human leukocyte antigens promote the surface expression of tumor antigens in cells (HLA-I). Antigen presentation must be successful at two different events to trigger an effective anti-tumor response: Dendritic cells (DCs) must first take up cancer antigens and cross-present them to CD8 + T-cell priming. Second, the tumor must directly expose the antigens for detection and destruction by primed CD8 + T cells [102]. In order to avoid immune detection at both of these processes, tumors utilize a variety of escape mechanisms.

The HPV responsible for CC has adopted some immunosuppressive strategies. They must modulate DC function to avoid the host's adaptive immunological response. Additionally, they affect epidermal DC recruitment and localization. The soluble regulatory factors produced by the hyperplastic epithelium of HPV alter DC formation and impact the initiation of particular cellular immune responses [103].

Immune evasion and T-cell trafficking

HPV is the primary cause of CC, with HPV16 and HPV18 accounting for 70% of cases. The host immune system normally eliminates HPV infection, but occasionally it persists because anti-HPV antibody synthesis is delayed. Thus, HPV develops machinery to evade the host immune system and is more persistent. 30% of CCs have been reported to reduce MHC I expression and upregulate MHC II. CXCL14 has been downregulated in HPV^+ CC, which helps recruit APCs, NK-cells, and T cells and prevent HPV-associated cancer progression. APCs show an immature phenotype in CC due to the downregulation of MHC co-stimulatory molecules CD80 and CD86. Thus, the DCs capacity is reduced to prime antigen-specific T cells. Likewise, the maturation of DCs is inhibited by IL-10, TGF-β, IL-6, PGE2, and GM-CSF. The expression of IDO1 is high in CC lesions. IDO1 promotes the development of Tregs, which are suppressors of anti-tumor immunity. The enzyme IDO1 negatively regulates anti-tumor immunity. The activity of NK cells is reduced with increased macrophage (TAMs) infiltration in CC. Th1 and Th2 response is seen in CC to secrete pro-inflammatory cytokines. Th17 overexpression relates to CC [104]. A study found many CD4 and CD25 cells recruited by Tregs related to HPV. Their presence is related to the stages of the disease [105]. In CC, there is downregulation of CXCL14 by promoter hypermethylation in an E7-dependent manner. Thus, there is immunosuppression of CD8 + T cells, which leads to a histone modification and downregulates TLR9 expression in HPV^+ CC [106].

Loss of antigen presentation and expression on immunomodulatory molecules such as PDL-1, CD155, CD74, and CD47

APCs help in antigen transportation from the periphery to lymphoid tissue. MHCs are required for antigen presentation and immune recognition. Dysregulation of MHC will cause immunotherapeutic resistance to tumors. Programmed death ligand 1 (PDL-1) is a well-known immune checkpoint that controls immune homeostasis. In CC, PDL-1 is overexpressed, which helps to evade the immune system. It happens when IFN-γ is released by activated T cells, which causes overexpression of PDL-1 in both tumor-infiltrating immune cells and tumor cells. By attaching to the B7-1 and PD-1 receptors on the surface of activated T cells, PDL-1 blocks the body's ability to fight cancer by deactivating the T cells' cytotoxic function. The binding of PDL-1 on tumor-infiltrating immune cells to B7-1 or PD-1 inactivates T cells. [107]. CD155 is a new immune checkpoint in cancer therapy. With a degree of CC, the expression of CD155 gradually increases. Downregulating it will inhibit cell proliferation, viability, and cell cycle arrest, restore CD8 + T cells, and produce cytokines. T-cell immunoglobulin and ITIM domain (TIGIT) binds with CD155, recruits SHIP-1, gets phosphorylated, and inhibits NF-κB and ERK activation. Thus, cytokine production is reduced. Blocking of TIGIT restores the function of CD8 + T cells. Knockdown of CD155 will inhibit AKT/mTOR/NF-κB pathway, activating autophagy and apoptosis [87, 107].

CD74 is a novel cell surface receptor for the cytokine migratory inhibitory factor (MIF) involved in forming and transporting MHC II for CD4 + T-cell response. Overexpression of CD74 inhibits MHC II leading to tumor metastasis. In CC, CD74 is overexpressed, making tumor-associated antigens (TAA) recognized by CD4 + T cells. It produces cytokines and inhibits tumor growth. CD74 coupled with CD44 induces phosphorylation on stimulation from MIF. It activates Src and ERK1/2 and dephosphorylates p53, inhibiting apoptosis [108]. CD47 is a transmembrane protein that binds to TSP-1 and SIRPα, protecting it from macrophages. In CC, it is overexpressed. CD47 on the tumor surface binds to SIRPα on the macrophage surface, preventing phagocytizing tumor cells. Blockade of CD47 activates CD8 + T cells. CD47-induced cell proliferation occurs via the PI3K/Akt pathway [109].

Role of computational biology in cervical cancer

With bioinformatics' rapid growth and development, protein structure prediction and thermodynamic features of target proteins can be investigated, which help find drug-binding sites and delineate drug action mechanisms [110]. Molecular docking is one of the most well-known and effective in silico techniques for predicting associations between molecules and biological targets. In a recent study, by combining docking and ligand-based approaches, Xu et al. could accurately anticipate the affinity and binding of several Hsp90 and farnesoid X receptor ligands [111]. In addition to docking, molecular dynamics and binding free energy estimates are frequently used to enhance the outcomes of virtual screening. They are particularly used to identify receptor conformations for docking [112]. A recent study showed that I1, an inhibitor of proliferating cell nuclear antigen (PCNA), binds to its hydrophobic pocket with great affinity. The study identified lead molecules that inhibit

the protein–protein interaction of PCNA, which might be a novel cancer therapeutic target [113]. Another study showed that MEK1 is an attractive cancer target due to its role in cell proliferation. Many MEK1 inhibitors so far are selective for many cancers but were found to be quite toxic with low efficiency. A study demonstrated that quinolines, an allosteric inhibitor of MEK1, gave satisfactory results with efficient binding affinity while being nontoxic [114]. Lately, approaches based on statistics and artificial intelligence (AI) have also made progress in drug discovery. In reality, these techniques make it simple to take advantage of the expanding body of knowledge found in structural, chemical, and bioactivity databases that are made available to the public, which allows predictions of binding affinity to be more precise [115]. Additionally, machine learning (ML) techniques have enhanced docking scoring functions. For instance, Ballester et al. created one of the first ML-based scoring functions, called "RF-Score," which employs Random Forest to improve predictions of protein–ligand binding affinity [116]. Computational biology has helped researchers utilize the vast molecular profiling data to define the fundamentals of tumor evolution and clarify how it manifests in various cancer types. The fatality rate of cancer is quite high as the cancer is detected at a late stage, but with the advent of AI and ML in medical science, cancer detection at an early stage and anticancer drug development have become possible [117]. A study showed that computer-aided drug designing inhibited tubulin's function, associated with uncontrolled cell division. The plant alkaloid etoposide was the best drug inhibiting tubulin function [118]. As HPV is considered a risk factor for cervical cancer, preventing the infection can greatly decrease the chances of developing cervical cancer. A study identified the 68 possible binding sites and 28 pockets in E6 oncoprotein on HVP16 by docking approach. From the receptor-ligand interaction profile, they identified that the amino acids, Leu50 and Cys51, were significant inhibitors for E6 oncoprotein [119]. AI can improve image classification and clarification of cancers. Kim et al. created a color-texture-based cervical image interpretation algorithm. They identified high-grade cervical tumors with 74% sensitivity and 90% specificity [120]. Cho et al. devised a binary decision model for cervical lesion biopsy. The RESNET-152 model had an average AUC of 0.947, 85.2% sensitivity, and 88.2% specificity, which helps inexperienced clinicians decide whether to conduct a cervical biopsy or send the patient to a specialist [121]. ML offers different algorithms used to evaluate the biochemical data of the drugs which speeds up the drug discovery process. Lind et al. used monitoring data and ML to produce synthetic data. The model was used to estimate how well anticancer drugs will work based on the location of a cancer cell's genome mutation [122] while Wang et al. developed an elastic net regression-based ML method for modeling drug susceptibility [123]. Precision oncology study may benefit from AI-based cancer diagnostics, stratification, mutation detection, therapy, and pharmaceutical repurposing techniques. Recent literature suggests that translational research examining this convergence will aid in resolving the most challenging issues facing precision medicine, particularly those in which nongenomic and genomic determinants will facilitate personalized diagnosis and prognostication when combined with data from patient symptoms, clinical history, and lifestyles [124]. A meta-analysis study demonstrated that artificial intelligence techniques could be effectively applied in personalized medicine and showed satisfactory results against cervical cancer [125]. AI and ML can quickly grasp how cancer cells develop resistance to cancer treatments by studying and analyzing data on severe drug-resistant cancers. This understanding will help to improve the development of new medications against cancer soon [126].

Future perspective and conclusion

In the clinic, patients suffering from or diagnosed with recurrent, progressive, and metastatic CC have a poor overall prognosis with an estimated survival of approximately one year. Bevacizumab and pembrolizumab represent two FDA-approved and vetted therapies; however, the success rate of complete response observed among these patients when challenged with these therapies alone is not very promising. The major reason is the rewiring of the intrinsic oncogenic pathways to trigger immune suppression to generate a hostile TME. An emphasis on the molecular causes of carcinogenesis in this age of precision medicine has resulted in a fresh arsenal of targeted therapies that have enhanced cancer treatment [127]. Recent years have seen an increase in interest in immunotherapy for CC due to a better knowledge of the interactions between HPV tumors and the host immune system and the emergence of novel therapies targeting immunological checkpoints. Immunotherapy will likely be a key component of managing locoregional, recurring, or metastatic CC, assuming that continuing investigations corroborate the promising findings of prior trials [128]. Combination regimens, including immune checkpoint inhibitors, DNA damage repair inhibitors, and antibody–drug conjugates, are being studied as potential ground-breaking therapy approaches [129]. In conjunction with metronomic chemotherapy, immune checkpoint inhibitors may offer a way to target therapy-resistant cells, such as CSCs and Tumor-initiating cells (TICs), without causing undesirable toxicity, leading to high medication adherence, improved long-term outcomes for challenging cancers, and enhanced patient quality of life [130]. With the advancement of modern technologies, such as spatial omics and single-cell genomic technology platforms, modern research should shed light on

the molecular biology of the TME of CC and demonstrate the spatial architecture of the cellular components within the TME. This knowledge will elaborate on ligand-receptor interaction and enable the discovery of novel biomarkers that may play an important role in predicting early recurrence. In addition, the information from these technologies will lead to alternative treatment options, possibly molecular targeted therapy in combination with immunotherapy. At the same time, other markers may help tailor therapy and attenuate the response to these novel therapies. Precise optimization will be highly warranted before translating these therapies into the clinic.

Acknowledgements The authors thank the Vellore Institute of Technology (VIT, University), Vellore, Tamil Nadu, India, for supporting this work.

Author contributions Conceptualization, GR, AVG, DS and GPDC. Resources and data curation, SD, AB, TM, writing—original draft preparation, AGM, URW, RM, and SK writing—review and editing, SD, AB, TM, AGM, URW, RM, and SK visualization, GR, AVG, DS and GPDC. Supervision, GR, AVG, DS and GPDC Project administration, DS and GPDC All authors have read and agreed to the published version of the manuscript.

Funding None.

Data availability Data are available from the authors on request (D.S.; G.P.D.C.).

Declarations

Conflicts of interest The authors declared no potential conflicts concerning this article's research, authorship, and publication.

References

1. Zhang S, et al. Cervical cancer: epidemiology, risk factors and screening. Chin J Cancer Res. 2020;32(6):720.
2. Patel C, et al. The impact of 10 years of human papillomavirus (HPV) vaccination in Australia: what additional disease burden will a nonavalent vaccine prevent? Euro Surveill. 2018;23(41):1700737.
3. Organization WH (2022) Cervical cancer. [Accessed 3 Nov 2022]. https://www.who.int/news-room/fact-sheets/detail/cervical-cancer
4. Lee C-L, et al. Standardization and experience may influence the survival of laparoscopic radical hysterectomy for cervical cancer. Taiwan J Obstet Gynecol. 2021;60(3):463–7.
5. Borges da Silva E, et al. Micronucleus assay for predicting side effects of radiotherapy for cervical cancer. Biotechnic Histochem. 2021;96(1):60–6.
6. Yadav N, Parveen S, Banerjee M. Potential of nano-phytochemicals in cervical cancer therapy. Clin Chim Acta. 2020;505:60–72.
7. Della Corte L, et al. Advances in paclitaxel combinations for treating cervical cancer. Expert Opin Pharmacother. 2020;21(6):663–77.
8. Zhao Y, et al. Tyrosine kinase inhibitors and their unique therapeutic potentialities to combat cancer. Int J Biol Macromol. 2021;168:22–37.
9. Guo Q, et al. Apatinib combined with chemotherapy or concurrent chemo-brachytherapy in patients with recurrent or advanced cervical cancer: a phase 2, randomized controlled, prospective study. Medicine. 2020;99(11):e91372.
10. Qiu H, et al. Apatinib, a novel tyrosine kinase inhibitor, suppresses tumor growth in cervical cancer and synergizes with Paclitaxel. Cell Cycle. 2018;17(10):1235–44.
11. Oaknin A, et al. Neratinib in patients with HER2-mutant, metastatic cervical cancer: findings from the phase 2 SUMMIT basket trial. Gynecol Oncol. 2020;159(1):150–156.
12. Burger RA. Role of vascular endothelial growth factor inhibitors in the treatment of gynecologic malignancies. J Gynecol Oncol. 2010;21(1):3–11.
13. Monk BJ, et al. Phase II, open-label study of pazopanib or lapatinib monotherapy compared with pazopanib plus lapatinib combination therapy in patients with advanced and recurrent cervical cancer. J Clin Oncol. 2010;28(22):3562–9.
14. Aydinlik S, Dere E, Ulukaya E. Induction of autophagy enhances apoptotic cell death via epidermal growth factor receptor inhibition by canertinib in cervical cancer cells. Biochim Biophys Acta. 2019;1863(5):903–16.
15. El-Meguid EAA, et al. Novel benzimidazole derivatives as anti-cervical cancer agents of potential multi-targeting kinase inhibitory activity. Arab J Chem. 2020;13(12):9179–95.
16. Zheng Y, Jiang Y. mTOR inhibitors at a glance. Mol Cell Pharmacol. 2015;7(2):15–20.
17. Wang X, Sun SY. Enhancing mTOR-targeted cancer therapy. Expert Opin Ther Targets. 2009;13(10):1193–203.
18. Assad DX, et al. Potential impact of mTOR inhibitors on cervical squamous cell carcinoma: a systematic review. Oncol Lett. 2016;12(5):4107–16.
19. Liu JJ, et al. Inhibition of phosphatidylinositol 3-kinase (PI3K) signaling synergistically potentiates anti-tumor efficacy of paclitaxel and overcomes paclitaxel-mediated resistance in cervical cancer. Int J Mol Sci. 2019;20(14):3383.
20. Schefter TE, et al. A phase II study of bevacizumab in combination with definitive radiotherapy and cisplatin chemotherapy in untreated patients with locally advanced cervical carcinoma: preliminary results of RTOG 0417. Int J Radiat Oncol Biol Phys. 2012;83(4):1179–84.
21. Tanigawa T, et al. Paclitaxel-carboplatin and bevacizumab combination with maintenance bevacizumab therapy for metastatic, recurrent, and persistent uterine cervical cancer: An open-label multicenter phase II trial (JGOG1079). Gynecol Oncol. 2022;165(3):413–9.
22. Zhang Y, et al. 7-Difluoromethyl-5, 4'-dimethoxygenistein inhibited the angiogenesis induced by cervical cancer SiHa cells via inhibiting TLR4/VEGF-A axis (217). Gynecol Oncol. 2022;166:S120–2.
23. Milosevic MF, et al. Sorafenib increases tumor hypoxia in cervical cancer patients treated with radiation therapy: results of a phase 1 clinical study. Int J Radiat Oncol Biol Phys. 2016;94(1):111–7.
24. Doll CM, et al. COX-2 expression and survival in patients with locally advanced cervical cancer treated with chemoradiotherapy and celecoxib: a quantitative immunohistochemical analysis of RTOG C0128. Int J Gynecol Cancer. 2013;23(1):176–83.
25. Gaffney DK, et al. A Phase II study of acute toxicity for Celebrex (celecoxib) and chemoradiation in patients with locally advanced cervical cancer: primary endpoint analysis of RTOG 0128. Int J Radiat Oncol Biol Phys. 2007;67(1):104–9.
26. Hefler LA, et al. The cyclooxygenase-2 inhibitor rofecoxib (Vioxx) in the treatment of cervical dysplasia grade II-III A phase II trial. Eur J Obstet Gynecol Reprod Biol. 2006;125(2):251–4.

27. Nogueira-Rodrigues A, et al. Phase 2 trial of erlotinib combined with cisplatin and radiotherapy in patients with locally advanced cervical cancer. Cancer. 2014;120(8):1187–93.
28. Goncalves A, et al. A phase II trial to evaluate gefitinib as second- or third-line treatment in patients with recurring locoregionally advanced or metastatic cervical cancer. Gynecol Oncol. 2008;108(1):42–6.
29. Jackson CG, et al. A phase II trial of bevacizumab and rucaparib in recurrent carcinoma of the cervix or endometrium. Gynecol Oncol. 2022;166(1):44–9.
30. Harkenrider MM, et al. Moving forward in cervical cancer: enhancing susceptibility to DNA repair inhibition and damage, an NCI clinical trials planning meeting report. J Natl Cancer Inst. 2020;112(11):1081–8.
31. Wieringa HW, et al. Breaking the DNA damage response to improve cervical cancer treatment. Cancer Treat Rev. 2016;42:30–40.
32. Kotsopoulos IC, et al. Poly(ADP-Ribose) polymerase in cervical cancer pathogenesis: mechanism and potential role for PARP inhibitors. Int J Gynecol Cancer. 2016;26(4):763.
33. IJff M, et al. PARP1-inhibition sensitizes cervical cancer cell lines for chemoradiation and thermoradiation. Cancers. 2021;13(9):2092.
34. Gupte R, et al. Combinatorial treatment with PARP-1 inhibitors and cisplatin attenuates cervical cancer growth through fos-driven changes in gene expression. Mol Cancer Res. 2022;20(8):1183–92.
35. Tewari KS, et al. Improved survival with bevacizumab in advanced cervical cancer. N Engl J Med. 2014;370(8):734–43.
36. Lightfoot M, Montemorano L, Bixel K. PARP inhibitors in gynecologic cancers: what is the next big development? Curr Oncol Rep. 2020;22(3):29.
37. Bozic I, Allen B, Nowak MA. Dynamics of targeted cancer therapy. Trends Mol Med. 2012;18(6):311–6.
38. Orbegoso C, Murali K, Banerjee S. The current status of immunotherapy for cervical cancer. Rep Pract Oncol Radiother. 2018;23(6):580–8.
39. Delavallee L, et al. Anti-cytokine vaccination in autoimmune diseases. Swiss Med Wkly. 2010;140: w13108.
40. Sahin U, Tureci O. Personalized vaccines for cancer immunotherapy. Science. 2018;359(6382):1355–60.
41. Faries MB, et al. Long-term survival after complete surgical resection and adjuvant immunotherapy for distant melanoma metastases. Ann Surg Oncol. 2017;24(13):3991–4000.
42. Surolia I, Gulley J, Madan RA. Recent advances in the use of therapeutic cancer vaccines in genitourinary malignancies. Expert Opin Biol Ther. 2014;14(12):1769–81.
43. Giaccone G, et al. A phase III study of belagenpumatucel-L, an allogeneic tumour cell vaccine, as maintenance therapy for non-small cell lung cancer. Eur J Cancer. 2015;51(16):2321–9.
44. Scholz M, et al. Phase I clinical trial of sipuleucel-T combined with escalating doses of ipilimumab in progressive metastatic castrate-resistant prostate cancer. Immunotargets Ther. 2017;6:11–6.
45. Khobragade A, et al. Efficacy, safety, and immunogenicity of the DNA SARS-CoV-2 vaccine (ZyCoV-D): the interim efficacy results of a phase 3, randomised, double-blind, placebo-controlled study in India. Lancet. 2022;399(10332):1313–21.
46. Morris VK, Jazaeri AA, Westin SN, Pettaway CA, George S, Huey R, Onstad M, Tu S-M, Wang J, Shafer A, Johnson B, Xiao L, Vining DJ, Guo M, Yuan Y, Frumovitz MM. Phase II trial of MEDI0457 and durvalumab for patients with recurrent/metastatic HPV-associated cancers. J Clin Oncol. 2021;39(15):2595–2595.
47. Huang J, et al. Isocitrate dehydrogenase mutations in glioma: from basic discovery to therapeutics development. Front Oncol. 2019;9:506.
48. Tregoning JS, et al. progress of the COVID-19 vaccine effort: viruses, vaccines and variants versus efficacy, effectiveness and escape. Nat Rev Immunol. 2021;21(10):626–36.
49. Bulcha JT, et al. Viral vector platforms within the gene therapy landscape. Signal Transduct Target Ther. 2021;6(1):53.
50. Galicia-Carmona T, et al. ADXS11-001 LM-LLO as specific immunotherapy in cervical cancer. Hum Vaccin Immunother. 2021;17(8):2617–25.
51. Kumar P. Recent advancement in cancer treatment. In: Design of nanostructures for theranostics applications. Amsterdam: Elsevier; 2018. p. 621–51.
52. Collignon C, et al. Innate immune responses to chimpanzee adenovirus vector 155 vaccination in mice and monkeys. Front Immunol. 2020;11: 579872.
53. Komdeur FL, et al. First-in-human phase I clinical trial of an SFV-based RNA replicon cancer vaccine against HPV-induced cancers. Mol Ther. 2021;29(2):611–25.
54. Maciag PC, Radulovic S, Rothman J. The first clinical use of a live-attenuated Listeria monocytogenes vaccine: a phase I safety study of Lm-LLO-E7 in patients with advanced carcinoma of the cervix. Vaccine. 2009;27(30):3975–83.
55. Skwarczynski M, Toth I. Peptide-based synthetic vaccines. Chem Sci. 2016;7(2):842–54.
56. Vici P, et al. Targeting immune response with therapeutic vaccines in premalignant lesions and cervical cancer: hope or reality from clinical studies. Expert Rev Vaccines. 2016;15(10):1327–36.
57. Durai V, Murphy KM. Functions of murine dendritic cells. Immunity. 2016;45(4):719–36.
58. Reuther S, et al. In vitro-induced response patterns of antileukemic T cells: characterization by spectratyping and immunophenotyping. Clin Exp Med. 2013;13(1):29–48.
59. Ferrara A, et al. Dendritic cell-based tumor vaccine for cervical cancer II: results of a clinical pilot study in 15 individual patients. J Cancer Res Clin Oncol. 2003;129(9):521–30.
60. Liu MA. DNA vaccines: a review. J Intern Med. 2003;253(4):402–10.
61. Youn JW, et al. Pembrolizumab plus GX-188E therapeutic DNA vaccine in patients with HPV-16-positive or HPV-18-positive advanced cervical cancer: interim results of a single-arm, phase 2 trial. Lancet Oncol. 2020;21(12):1653–60.
62. Melero I, et al. Evolving synergistic combinations of targeted immunotherapies to combat cancer. Nat Rev Cancer. 2015;15(8):457–72.
63. Mitra A, et al. Cervical intraepithelial neoplasia: screening and management. Br J Hosp Med (Lond). 2016;77(8):C118–23.
64. Miles B, Safran HP, Monk BJ. Therapeutic options for treatment of human papillomavirus-associated cancers—novel immunologic vaccines: ADXS11-001. Gynecol Oncol Res Pract. 2017;4:10.
65. Miller RL, Meng TC, Tomai MA. The antiviral activity of Toll-like receptor 7 and 7/8 agonists. Drug News Perspect. 2008;21(2):69–87.
66. Bilu D, Sauder DN. Imiquimod: modes of action. Br J Dermatol. 2003;149(Suppl 66):5–8.
67. Feng CH, et al. Immunotherapy with radiotherapy and chemoradiotherapy for cervical cancer. Semin Radiat Oncol. 2020;30(4):273–80.
68. Kanaan H, Kourie HR, Awada AH. Are virus-induced cancers more sensitive to checkpoint inhibitors? Future Oncol. 2016;12(23):2665–8.
69. Jean-Sebastien F, Christoph LT, Oeil BH, Patrick AO, Piha-Paul SA, Gomez-Roca CA, Van Brummelen E, Rugo HS, Thomas

S, Saraf S, Chen M, Varga A. Pembrolizumab in patients with advanced cervical squamous cell cancer: preliminary results from the phase Ib KEYNOTE-028 study. J Clin Oncol. 2016;34(15):5515–5515.
70. Frenel JS, et al. Safety and efficacy of pembrolizumab in advanced, programmed death ligand 1-positive cervical cancer: results from the phase Ib KEYNOTE-028 trial. J Clin Oncol. 2017;35(36):4035–41.
71. Naumann RW, et al. Safety and efficacy of nivolumab monotherapy in recurrent or metastatic cervical, vaginal, or vulvar carcinoma: results from the phase I/II CheckMate 358 trial. J Clin Oncol. 2019;37(31):2825–34.
72. O'Malley DM, Monk BJ, Leary A, Selle F, Alexandre J, Randall LM, Rojas C, Neffa M, Kryzhanivska A, Gladieff L, Berton D, Meniawy T, Lugowska I, Bondarenko I, Moore KN, OrtuzarFeliu WI, Ancukiewicz M, Shapiro I, Ray-Coquard IL. LBA34 single-agent anti-PD-1 balstilimab or in combination with anti-CTLA-4 zalifrelimab for recurrent/metastatic (R/M) cervical cancer (CC): preliminary results of two independent phase II trials. Ann Oncol. 2020;31:1164–5.
73. Jan HM, Schellens AM, Susan Z, Jie D, Scott KP, Hyun CC. Pembrolizumab for previously treated advanced cervical squamous cell cancer: preliminary results from the phase 2 KEYNOTE-158 study. J Clin Oncol. 2017;35(15):5514–5514.
74. Jeffrey C, Patel R, Hasan R, Shreya G, Saif MW. Recent advances in immunotherapy for pancreatic cancer. J Cancer Metastas Treat. 2020;6:43.
75. Khalil A, Kamar A, Nemer G. Thalidomide-revisited: are COVID-19 patients going to be the latest victims of yet another theoretical drug-repurposing? Front Immunol. 2020;11:1248.
76. Semeraro M, et al. Trial Watch: Lenalidomide-based immunochemotherapy. Oncoimmunology. 2013;2(11): e26494.
77. Wang Y, et al. CD133-directed CAR T cells for advanced metastasis malignancies: A phase I trial. Oncoimmunology. 2018;7(7): e1440169.
78. Stevanovic S, et al. Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells. J Clin Oncol. 2015;33(14):1543–50.
79. Lu YC, Lu T, Zheng Z, Toomey MA, White DE, Yao X, Li YF, Robbins PF, Feldman SA, van der Bruggen P, Klebanoff CA, Goff SL, Sherry RM, Kammula US, Yang JC, Rosenberg SA. Treatment of patients with metastatic cancer using a major histocompatibility complex class II-restricted T-cell receptor targeting the cancer germline antigen MAGE-A3. J Clin Oncol. 2017;35(29):3322–9.
80. Talens F, Van Vugt M. Inflammatory signaling in genomically instable cancers. Cell Cycle. 2019;18(16):1830–48.
81. Sharma P, et al. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell. 2017;168(4):707–23.
82. Muller E, et al. Genetic profiles of cervical tumors by high-throughput sequencing for personalized medical care. Cancer Med. 2015;4(10):1484–93.
83. Husain RS, Ramakrishnan V. Global variation of human papillomavirus genotypes and selected genes involved in cervical malignancies. Ann Glob Health. 2015;81(5):675–83.
84. Wingo SN, et al. Somatic LKB1 mutations promote cervical cancer progression. PLoS ONE. 2009;4(4): e5137.
85. Salk JJ, Kennedy SR. Next-generation genotoxicology: using modern sequencing technologies to assess somatic mutagenesis and cancer risk. Environ Mol Mutagen. 2020;61(1):135–51.
86. Bahrami A, et al. The potential value of the PI3K/Akt/mTOR signaling pathway for assessing prognosis in cervical cancer and as a target for therapy. J Cell Biochem. 2017;118(12):4163–9.
87. Zhu L, et al. A narrative review of tumor heterogeneity and challenges to tumor drug therapy. Ann Transl Med. 2021;9(16):1351.
88. Yao Y, Dai W. Genomic instability and cancer. J Carcinog Mutagen. 2014;5:1000165.
89. Liu YP, et al. Molecular mechanisms of chemo- and radiotherapy resistance and the potential implications for cancer treatment. MedComm. 2021;2(3):315–40.
90. Arts P, et al. Exome sequencing in routine diagnostics: a generic test for 254 patients with primary immunodeficiencies. Genome Med. 2019;11(1):38.
91. Manzo-Merino J, et al. The role of signaling pathways in cervical cancer and molecular therapeutic targets. Arch Med Res. 2014;45(7):525–39.
92. Gutierrez-Hoya A, Soto-Cruz I. Role of the JAK/STAT pathway in cervical cancer: its relationship with HPV E6/E7 oncoproteins. Cells. 2020;9(10):2297.
93. Al-Amrani S, et al. Proteomics: Concepts and applications in human medicine. World J Biol Chem. 2021;12(5):57–69.
94. de la Cruz-Lopez KG, et al. Lactate in the regulation of tumor microenvironment and therapeutic approaches. Front Oncol. 2019;9:1143.
95. Palan PR, et al. Plasma levels of antioxidant beta-carotene and alpha-tocopherol in uterine cervix dysplasias and cancer. Nutr Cancer. 1991;15(1):13–20.
96. Potischman N, Brinton LA. Nutrition and cervical neoplasia. Cancer Causes Control. 1996;7(1):113–26.
97. Sica A. Role of tumour-associated macrophages in cancer-related inflammation. Exp Oncol. 2010;32(3):153–8.
98. Sica A, et al. Macrophage polarization in tumour progression. Semin Cancer Biol. 2008;18(5):349–55.
99. Yuan Y, et al. role of the tumor microenvironment in tumor progression and the clinical applications (Review). Oncol Rep. 2016;35(5):2499–515.
100. Ko YH, et al. Glutamine fuels a vicious cycle of autophagy in the tumor stroma and oxidative mitochondrial metabolism in epithelial cancer cells: implications for preventing chemotherapy resistance. Cancer Biol Ther. 2011;12(12):1085–97.
101. Efremova M, et al. Neoantigens generated by individual mutations and their role in cancer immunity and immunotherapy. Front Immunol. 2017;8:1679.
102. Kwiatkowski N, et al. Targeting transcription regulation in cancer with a covalent CDK7 inhibitor. Nature. 2014;511(7511):616–20.
103. Bashaw AA, et al. Modulation of antigen presenting cell functions during chronic HPV infection. Papillomavirus Res. 2017;4:58–65.
104. Zhou C, Tuong ZK, Frazer IH. Papillomavirus immune evasion strategies target the infected cell and the local immune system. Front Oncol. 2019;9:682.
105. Zhang L, et al. A review of the research progress in T-lymphocyte immunity and cervical cancer. Transl Cancer Res. 2020;9(3):2026–36.
106. Westrich JA, Warren CJ, Pyeon D. Evasion of host immune defenses by human papillomavirus. Virus Res. 2017;231:21–33.
107. Liu Y, et al. PD-1/PD-L1 inhibitors in cervical cancer. Front Pharmacol. 2019;10:65.
108. Balakrishnan CK, et al. CD74 and HLA-DRA in cervical carcinogenesis: potential targets for antitumour therapy. Medicina (Kaunas, Lithuania). 2022;58(2):190.
109. Xu S, et al. LSD1 silencing contributes to enhanced efficacy of anti-CD47/PD-L1 immunotherapy in cervical cancer. Cell Death Dis. 2021;12(4):282.
110. Lin X, Li X, Lin X. A review on applications of computational methods in drug screening and design. Molecules. 2020;25(6):1375.
111. Xu X, Yan C, Zou X. Improving binding mode and binding affinity predictions of docking by ligand-based search of protein

conformations: evaluation in D3R grand challenge 2015. J Comput Aided Mol Des. 2017;31(8):689–99.
112. Salmaso V, Moro S. Bridging molecular docking to molecular dynamics in exploring ligand-protein recognition process: an overview. Front Pharmacol. 2018;9:923.
113. Bhardwaj VK, Purohit R. A lesson for the maestro of the replication fork: targeting the protein-binding interface of proliferating cell nuclear antigen for anticancer therapy. J Cell Biochem. 2022;123(6):1091–102.
114. Singh R, Bhardwaj VK, Purohit R. Computational targeting of allosteric site of MEK1 by quinoline-based molecules. Cell Biochem Funct. 2022;40(5):481–90.
115. Lavecchia A. Machine-learning approaches in drug discovery: methods and applications. Drug Discov Today. 2015;20(3):318–31.
116. Ballester PJ, Mitchell JB. A machine learning approach to predicting protein-ligand binding affinity with applications to molecular docking. Bioinformatics. 2010;26(9):1169–75.
117. Vobugari N, et al. Advancements in oncology with artificial intelligence: a review article. Cancers (Basel). 2022;14(5):1349.
118. Yadav M, Dhagat S, Eswari JS. Structure based drug design and molecular docking studies of anticancer molecules paclitaxel, etoposide and topotecan using novel ligands. Curr Drug Discov Technol. 2020;17(2):183–90.
119. Kolluru S, et al. Identification of potential binding pocket on viral oncoprotein HPV16 E6: a promising anti-cancer target for small molecule drug discovery. BMC Mol Cell Biol. 2019;20(1):30.
120. Kim E, Huang X. A data driven approach to cervigram image analysis and classification. Color medical image analysis. In: Celebi SG, editor. Lecture notes in computational vision and biomechanics. Dordrecht: Springer; 2013.
121. Cho BJ, et al. Classification of cervical neoplasms on colposcopic photography using deep learning. Sci Rep. 2020;10(1):13652.
122. Li X, et al. Application of artificial intelligence in the diagnosis of multiple primary lung cancer. Thorac Cancer. 2019;10(11):2168–74.
123. Wang Y, et al. Systematic identification of non-coding pharmacogenomic landscape in cancer. Nat Commun. 2018;9(1):3192.
124. Johnson KB, et al. Precision medicine, AI, and the future of personalized health care. Clin Transl Sci. 2021;14(1):86–93.
125. Rezayi S, Saeedi S. Effectiveness of artificial intelligence for personalized medicine in neoplasms: a systematic review. Biomed Res Int. 2022;2022:7842566.
126. Dlamini Z, et al. Artificial intelligence (AI) and big data in cancer and precision oncology. Comput Struct Biotechnol J. 2020;18:2300–11.
127. Crusz SM, Miller RE. Targeted therapies in gynaecological cancers. Histopathology. 2020;76(1):157–70.
128. Attademo L, et al. Immunotherapy in cervix cancer. Cancer Treat Rev. 2020;90: 102088.
129. Mutlu L, et al. Targeted treatment options for the management of metastatic/persistent and recurrent cervical cancer. Expert Rev Anticancer Ther. 2022;22:633–45.
130. Kareva I. A combination of immune checkpoint inhibition with metronomic chemotherapy as a way of targeting therapy-resistant cancer cells. Int J Mol Sci. 2017;18(10):2134.