Emerging Therapeutic Agents for Cervical Cancer

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Abstract: Cervical cancer is the second most frequent malignancy affecting women worldwide. The highest incidences occur in the developing world, where, in most countries, cervical cancer is the leading cause of cancer mortality in women. Although surgery and chemoradiotherapy can cure 80-95% of women with early stage cancer and 60% of locoregionally advanced cancer, the recurrent and metastatic disease remains a major cause of cancer death. The current cytotoxic treatment options for advanced and metastatic cancer demonstrate modest results, with response rates of maximum 30% and overall survival of less than 10 months. Given this limited degree of success with conventional therapies, interest has increased in other therapeutic alternatives. In this way, targeted agents are emerging as potential candidates for improving survival in cervical cancer patients. In this review we highlight the main current therapeutic strategies for cervical cancer and summarize the most relevant patents from the latest five years. Special attention was given to patents with potential applications in the clinical practice.

Keywords: Targeted therapy, cervical cancer, growth factors.

INTRODUCTION

Cervical cancer is the second most frequent malignancy affecting women worldwide, with approximately 500,000 new cases diagnosed and 280,000 deaths each year [1]. The highest incidences occur in the developing world, where, in most countries, cervical cancer is the leading cause of cancer mortality in women [2]. Although surgery and chemoradiotherapy can cure 80-95% of women with early stage cancer and 60% of locoregionally advanced cancer, the recurrent and metastatic disease remains a major cause of cancer death [3]. The current cytotoxic treatment options for advanced and metastatic cancer demonstrate modest results, with response rates of maximum 30% and overall survival of less than 10 months [4]. Given this limited degree of success with conventional therapies, interest has increased in other therapeutic alternatives. In this way, targeted agents are emerging as potential candidates for improving survival in cervical cancer patients. This review article will highlight the current therapeutic strategies and the most recent patents for cervical cancer treatment.

EARLY-STAGE DISEASE

In developed countries, where screening for cervical cancer is effective, approximately half of the patients present with stage I disease at the time of diagnosis. For these patients there are a number of acceptable treatment options that are based on surgery and/or radiation therapy. Most retrospective studies suggest that radical hysterectomy and pelvic radiation therapy are equally effective for the treatment of stage IB1. As tumor size increases, there is a higher risk for treatment failure. In this way, additional therapeutic modalities are being included for patients with stage IB2 disease. For patients stage IB2 who undergo radiotherapy, concurrent cisplatin-based chemotherapy has proven to improve overall and progression-free survival. Based on these results, chemotherapy associated to radiation is being considered the standard treatment for early-stage bulky tumors [5-8]. Neoadjuvant chemotherapy is also being employed for some groups of patients, including stage IB2 [9]. Randomized trials have suggested advantage in survival with the use of chemotherapy prior to radical pelvic surgery [10, 11]. Results from meta-analysis, however, are conflicting. While some authors found no survival advantage using neoadjuvant chemotherapy [12], other encountered a significant decrease in the risk of death from cervical cancer with this approach [13]. Additional controlled studies are needed to incorporate the neoadjuvant chemotherapy in the therapeutic arsenal for early-stage cervical cancer.

LOCALLY ADVANCED DISEASE

The patients who present with advanced lesions at diagnosis are at greater risk of recurrence and account for the majority of cervical cancer deaths. The treatment of choice for locally advanced tumors (stages IIA-IVA) has usually been based on pelvic external-beam radiation or intracavitary

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brachitherapy. However, radiotherapy alone fails to control progression of cervical cancer in 35% to 90% of women with locally advanced disease. As in many other solid malignancies, the addition of concurrent chemotherapy has been employed in attempt to minimize the risk of recurrence and distant metastasis. In cervical carcinomas, several randomized phase III trials have shown overall survival advantage for cisplatin-based therapy given concomitant with radiation therapy. The risk of death from cervical cancer decreased by 30% to 50% with the use combined chemoradiotherapy. Based on these results, there is a strong recommendation for adjuvant cisplatin-based chemotherapy for patients who undergo radiotherapy [5-8, 14, 15].

Whether cisplatin is more effective as a single agent or in combination with other drugs is currently under investigation [15, 16]. The GOG Trial of pelvic radiotherapy plus concurrent single-agent cisplatin versus cisplatin plus FU plus hydroxyurea versus hydroxyurea alone showed significant improvements in progression-free and overall survival in patients randomly assigned for either cisplatin-containing arm. In this study, cisplatin alone was equally effective and less toxic than the three-drug regimen [15]. A second GOG trial tested cisplatin and hydroxyurea as single agents and cisplatin followed by FU concomitant to radiotherapy for patients with stages IIB to IVA. Again, survival rates were higher in both cisplatin-containing regimens [16]. Carboplatin, a platin derivative which is associated with less toxicity than cisplatin, has been investigated as a radiation-sensitizing agent in advanced cervical cancer. In phase I and II studies, carboplatin at a weekly schedule demonstrated to be effective and safe [17, 18]. Although this is a promising drug for cervical cancer treatment, phase III studies should be conducted comparing carboplatin with cisplatin during radiotherapy.

Other chemotherapeutic agents have been studied for advanced disease as well. In a general way, their performance as single agents is poor, but in combination with cisplatin the effectiveness is increased. The Taiwanese trial randomly assigned women with bulky IIB or IIIB cervical cancer to radiotherapy with or without concurrent multiagent chemotherapy (cisplatin, vinblastine and bleomycin). In this study, chemotherapy did not improve overall survival or disease-free survival after a median 47 month follow-up [19]. Paclitaxel in association to carboplatin was tested in a phase II trial that included women stages IB to IVA cervical cancer who undergone radiotherapy. The 3-year overall survival rates were 91%, 88% and 50% for stages IIB, III and IV, respectively [20]. Gemcitabine alone concomitant to radiotherapy showed elevated response rates at very low toxicity in a phase I study among patients with advanced cervical carcinoma [21]. Gemcitabine was further investigated as a radiosensitizer in a phase III trial in comparison to cisplatin. Even though the toxicity and overall response rates were similar among both agents, the complete responses were higher in the gemcitabine group [22]. The addition of topotecan to cisplatin during pelvic irradiation for locally advanced cervical cancer led to a complete response rate of 92% in a recent phase I trial [23]. Recent data suggest that topotecan, when used concurrently with cisplatin, may be the new standard of care for the management of advanced cervical cancer. Ongoing phase III studies will compare this combination with other cisplatin-containing and cisplatin-free combinations [24]. Although these and other combinations are promising regimens for advanced carcinoma of the cervix, weekly cisplatin in association to radiotherapy remains the standard of care.

RECURRENT AND DISSEMINATED DISEASE

Recurrent and disseminated cervical cancers are associated with poor survival rates. The prognosis in recurrent disease depends on the site of recurrence and the ability to pursue potentially curative therapy, among other factors. In locally recurrent tumors, pelvic exenteration can lead to a 5-year survival rate of 32% to 62% in selected patients [25]. Salvage radiotherapy may be an option for some patients if it has not been administered before.

Once the disease is spread beyond the confines of a radiation or surgical field, no standard treatment is available. In these cases, the main objective is palliation of symptoms. Radiotherapy may be useful to relieve pelvic pain or bleeding from advanced lesions. In metastatic disease, radiation can control pain from skeletal metastases or symptoms related to brain lesions [26].

Patients under palliative treatment should be candidates for single agent or combined chemotherapy. In a general way, the single agent approach is reserved for women with poor health, who would not tolerate combination chemotherapy. Several single chemotherapy agents and combination regimens have demonstrated activity in metastatic disease or in recurrences not amenable to local therapy. Cisplatin is to date the most active drug against cervical cancer, with response rates that range from 18% to 38% [27]. As renal and gastrointestinal toxicities increase proportionately to higher doses of cisplatin, other platinum derivatives have been studied with the intent of obtaining lower toxicity levels. Carboplatin at a dose of 400mg/m^2 every 4 weeks produced response rates as high as 28% and median survival of 6 to 7 months [28]. Iproplatin was studied in a randomized phase III trial of advanced cervical carcinomas, showing poorer response rates (10,8% versus 15,4%) in comparison to carboplatin [29]. Other classes of chemotherapeutic agents have shown activity in recurrent and metastatic cervical cancer, among them paclitaxel, topotecan, vinorelbine and ifosfamide [30]. Nevertheless, no single agent proved to be superior to cisplatin. In this way, research is being directed to combining agents with cisplatin or comparing combination therapy to single-agent cisplatin in randomized controlled trials.

The addition of active agents to cisplatin generally results in higher response rates, although the benefit in prolonged survival is small. The patients who have better response rates are those naïve of prior treatments. Many agents have been investigated in phase III trials in combination to cisplatin, including gemcitabine, vinorelbine, topotecan, ifosfamide, paclitaxel, fluoracil and bleomycin. The great majority demonstrated a response advantage over single-agent cisplatin [30]. Ifosfamide [31], paclitaxel [32] and topotecan [33] showed progression-free survival advantages compared to cisplatin alone. Regarding overall survival, topotecan is the only agent in combination to cisplatin that demonstrated advantage in a controlled trial [33].

In attempt to achieve even better response rates, multidrug chemotherapy has been tested for recurrent and metastatic cervical cancer. Several triplets combining active agents with cisplatin have demonstrated response rates of more than 50% and survival rates slightly superior to single-agent or doublet regimens in phase II studies. However, this benefit has not been confirmed in phase III trials; no triplet has proven greater activity than cisplatin as single-agent or in a doublet regimen [34-38].

TARGETED THERAPIES

In the last decade, several molecular events in cervical carcinogenesis have been elucidated, leading to the development of targeted agents for both diagnostic and therapeutic purposes. Noteworthy, the epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) have been widely studied in many solid malignancies. In cervical cancer, anti-EGFR and anti-VEGF therapies have been evaluated, in one effort to improve the limited results from current cytotoxic therapies.

Anti-EGFR Therapies

The EGF family of tyrosine kinases receptors is divided is four members: EGFR (HER1), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4) [39]. EGFR is highly expressed in primary and recurrent cervical tumors, and this expression has been correlated to poor prognosis and to more advanced stages of disease [40-42]. Additionally, EGFR has been shown to modulate chemosensitivity and radiosensitivity in pre-clinical trials [43, 44]. The EGFR has been successfully targeted either through monoclonal antibodies (cetuximab) or through small molecules inhibitors of tyrosine kinase (erlotinib and gefitinib).

Cetuximab, a chimerized antibody of the immunoglobulin G1 subclass, is highly specific for EGFR and has proven activity as monotherapy or in adjuvance to chemotherapy in head and neck, colorectal and lung cancers [45]. Based on these results, cetuximab has been tested as single agent or combined to radiotherapy and chemotherapy for cervical cancer treatment. In experimental studies, cetuximab demonstrated notable cellular toxicity and tumor growth inhibition [46]. Results from ongoing clinical trials with cetuximab are awaited, among them a phase II trial of cetuximab plus cisplatin as first-line chemotherapy for persistent or recurrent cervical carcinoma (GOG-0076DD), a study of cetuximab as monotherapy for recurrent or persistent cervical carcinoma (GOG-0227E) and a study of cetuximab in adjuvance to radiotherapy in early-stage disease (GOG-9918). Cetuximab might be a novel and attractive therapeutic strategy in patients harboring chemotherapy-resistant, recurrent, or metastatic cervical cancer.

EGFR tyrosine kinase inhibitors are also being studied for cervical malignancies. Gefitinib was investigated in a phase II clinical trial as second- and third-line single agent for recurrent squamous or adenocarcinoma of the cervix. No objective responses were found, though 20% of the patients had disease stabilized, with a median duration of stable disease of approximately 100 days [47]. Erlotinib, another specific EGFR tyrosine kinase inhibitor, is currently being tested in combination with radiotherapy and chemotherapy for locally advanced cervical cancer (NCT00428194) and as a single agent for persistent or recurrent disease (GOG-0227D). In a phase I trial, erlotinib in combination to cisplatin and pelvic radiotherapy was well tolerated in patients with locally advanced cervical tumors [48]. Dai and colleagues found that acquired resistance to cytotoxic therapy in cervical cancer cell lines was associated with enhanced sensitivity to erlotinib, which correlated with increased EGFR expression [49]. The authors suggested that EGFR tyrosine kinase inhibitors might be more effective as second- or third-line treatment for certain patients with tumors that were previously treated with multiple chemotherapy regimens. Lapatinib, another tyrosine kinase inhibitor which targets both EGFR and HER2, is being evaluated as monotherapy in a phase II clinical trial for locally advanced (IVB), persistent or recurrent cervical cancer (VEG105281).

Anti-VEGF Therapies

VEGF signaling is an attractive target for cancer therapy given its role in tumor angiogenesis and in endothelial cancer cell proliferation, differentiation, survival and migration. The overexpression of VEGF is usually related to poor prognosis and progression of cervical carcinomas [50]. Additionally, increased VEGF expression and tumor vascularization can be independent predictors of poor disease-free and overall survival [51]. Currently, the most studied anti-VEGF agent is bevacizumab, a humanized immunoglobulin G1 monoclonal antibody that binds to VEGF. In experimental studies, bevacizumab inhibited VEGF-induced proliferation of endothelial cells and decreased microvessel density in tumor xenografts [50]. Bevacizumab in addition to chemotherapy significantly improved overall survival in patients with metastatic colorectal and non-small lung cancer. These favorable results provided a rationale for testing this agent for cervical cancer treatment. The use of bevacizumab in heavily pretreated women with recurrent cervical carcinoma demonstrated clinical benefit in 67% of the patients, which included 1 (17%) complete response, 1 (17%) partial response and two (33%) patients with stable disease. The median time to progression for the women who demonstrated clinical benefit was 4.3 months [52].

Other Potential Targets

Beyond EGFR and VEGF, many other targets have been investigated for cervical cancer treatment. The phosphatidylinositol 3-kinase (PI3K) signaling pathway has been implicated in cervical carcinogenesis. PI3K was shown to be over expressed in cervical tumors, but not in normal tissues. Through cell lines experiments, a PI3K inhibitor, LY29400, significantly inhibited Hela cells growth and induced apoptosis [53]. LY29400 has also been tested as a radiosensitizer in cervical cancer cells. Although LY294002 alone did not produce cytotoxic effects, PI3K inhibition with this antagonist produced significant radiosensitization, showed significant time-dependent effects, increased apoptosis, and altered gene expression [54].

The heat shock protein 90 (Hsp90) is a conserved chaperone involved in crucial signaling events in normal and malignant cells. It is believed that tumor cells are particularly

dependent on Hsp90 for survival as well as for malignant progression. Hsp90 inhibitors, which are derivates of the natural compound geldanamycin, such as the orally bioavailable 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG), are currently being tested in clinical trials and small molecule inhibitors are in development. Through *in vitro* experiments, some researchers evaluated the effect of 17-DMAG in a panel of cervical carcinoma cell lines and demonstrated that Hsp90 inhibition effectively induced apoptosis and growth arrest [55]. HSP90 has also been identified as a molecular target for ionizing radiation. The treatment of two human cervical carcinoma cell lines (Hela and SiHa) with geldanamycin and its 17-allylamino-17-demethoxy analog (17-AAG) resulted in cytotoxicity and, when combined with ionizing radiation, enhanced the radiation response [56].

Cyclin-dependent kinases (CDK) play a crucial role in the control of the cell cycle. Recently, inhibition of CDKs by pharmacological inhibitors became a promising therapeutic option. Roscovitine, a selective CDK inhibitor, is known to efficiently target human malignant cells and induce cell cycle arrest and apoptosis through activation of p53 tumor suppressor protein. This effect was demonstrated in cervical cancer as well. Through *in vitro* studies with Hela cells, Roscovitine induced site-specific phosphorylation of p53 protein and apoptosis [57]. Another CDK inhibitor, NU6140, was tested in Hela cells, alone or in association with paclitaxel, with respect to apoptosis, inhibition of cell proliferation and cell cycle progression. Results from this study indicated that NU6140 significantly potentiated the apoptotic effect of paclitaxel, with inhibition of survivin expression/phosphorylation as the potential mechanism [58].

Finally, another interesting target is the Notch signal transduction pathway. In the latest years, it has been established that the Notch pathway mediates cell differentiation and proliferation. Intracellular forms of Notch1 have been detected in human cervical cancers, and its signaling pathway seems to complement the function of papillomavirus oncogenes. The activation of PI3K/Akt pathway and the up regulation of c-Myc have been proposed by some researchers as possible pro-oncogenic effector mechanisms [59]. Wang and colleagues showed that the overexpression of active Notch1 inhibited cervical carcinomas cells growth through induction of cell cycle arrest. Increased Notch1 signaling induced a downmodulation of human papillomavirus transcription through suppression of activator protein (AP)-1 activity by upregulation of c-Jun and the decreased expression of c-Fos [60]. In another study that used RNA interfering vectors to construct a course recombination enzyme-dependent short hairpin RNA expression plasmid targeting Notch1 in Hela cells, Notch1 expression was inhibited, intracellular Notch1 signal level was decreased and the cellular proliferation was suppressed [61].

Table **1** summarizes the current therapeutic options and the new perspectives for cervical cancer treatment according to FIGO stage.

RECENT PATENTS FOR CERVICAL CANCER TREATMENT

US20080171051 - Cancer Treatment

In this invention the combined treatment of a death receptor ligand, such as an anti FAS antibody, with a chemotherapeutic agent like 5-FU or an antifolate drug, produces a synergistic effect in killing cancer cells. However, the synergistic effect achieved is abrogated in cancer cells which overexpress c-FLIP. In cell lines which demonstrate overexpression of c-FLIP and associated resistance to chemotherapy induced apoptosis, inhibition of FLIP expression reversed the resistance to chemotherapy-induced apoptosis.

Table 1. Current Therapeutic Options and New Perspectives for Cervical Cancer Treatment

FIGO Stage	Current Therapeutic Options	New Perspectives
IA - IB1 IIA (<4cm)	S or RT	
IB2 IIA (> 4cm)	S ± adjuvant RT CT (cisplatin) + RT ± adjuvant S	Neoadjuvant CT + S ± RT Combination CT + RT Other CT agents* Biologic agents**
IIB - IVA	CT (cisplatin) + RT	Neoadjuvant CT Combination CT + RT Other CT agents* Biologic agents**
IVB	Palliative CT (cisplatin)	Combination CT Other CT agents* Biologic agents**

CT: Chemotherapy; RT: Radiotherapy; S: Surgery.

*including carboplatin, nedaplatin, paclitaxel, gemcitabine, capecitabine, vinorelbine, ifosfamide and topotecan.

** including cetuximab, bevacizumab, erlotinib, gefitinib, lapatinib, sorafenib and celecoxib.

On further investigating this effect, the inventors tested a number of cell lines having a p53 mutation or p53 null genotype, and observed that down-regulation of c-FLIP markedly enhanced apoptosis in response to certain chemotherapeutic agents. This observation led to the invention of c-FLIP inhibi-tors combined to chemotherapeutic agents for the treatment of malignancies associated with p53 mutations, among them cervical cancer.

The c-FLIP inhibitor and the chemotherapeutic agent (thymidylate synthase inhibitor, platinum cytotoxic agent or topoisomerase inhibitor) may be provided and administered in the absence of other active agents. However, in a preferred embodiment of theses aspects of the invention, there is provided a death receptor binding member, or a nucleic acid encoding said binding member. Any suitable death receptor binding member may be used. Death receptors include Fas, TNFR, DR-3, DR-4 and DR-5. Preferably, the c-FLIP inhibitor and the chemotherapeutic agent are administered in a potentiating ratio, such that the cytotoxic activity of the combination is greater than that of either component alone or of the additive activity that would be predicted for the combinations based on the activities of the individual components. Thus in a potentiating ratio, the individual components act synergistically. The c-FLIP inhibitor can be an RNAi agent, which modulates expression of the c-FLIP gene, or a siRNA, a shRNA, a ddRNAi construct or a transcription template thereof, e.g., a DNA encoding a shRNA [62].

US20080113340 - Diagnosis and Treatment of Cervical Cancer

In US20080113340 the invention relates to methods of diagnosing cervical diseases or conditions, including cervical cancer, cervical precancerous lesions, or immortalization of cervical cells, by using a panel of biomarkers. The invention also relates to methods of treating cervical diseases by targeting one or more of these biomarkers.

The diagnosing method comprises analyzing the status of at least two of the following biomarkers: human telomerase reverse transcriptase (hTERT), insulin-like growth factor binding protein 3 (IGFBP-3), transferrin receptor, beta-catenin, Myc-human papilloma virus (HPV) E6 interaction, HPV E7, and telomere length, in cervical cells of the female. If the biomarker is hTERT, IGFBP-3, transferrin receptor or HPV E7, the status to be assessed is the expression level of the biomarker. Preferably, the expression level of HPV E7 is analyzed by flow cytometry. Increased expression level of the biomarker relative to an appropriate control level (e.g., obtained from a healthy female) indicates that the female has cervical cancer or is at increased risk of developing this tumor. If the biomarker is beta-catenin, the status to be assessed is the level and localization of beta-catenin in the cytoplasm and/or nucleus. In case the biomarker is Myc-HPV E6 interaction, the association between Myc and HPV E6 is analyzed. Further on, if the biomarker is telomere length, an increased telomere length relative to control indicates that the female has cervical carcinoma or has a higher risk of developing it.

The invention also provides a method of classifying the grade of a cervical lesion for diagnostic and/or prognostic purposes. Such method aims to determinate the status of one or more biomarkers (including hTERT, IGFBP-3, transferrin receptor, beta-catenin, Myc-HPV E6 interaction, HPV E7, and telomere length, and combinations) in a cervical cell of a female to provide an individual biomarker diagnostic for cervical lesions. The status of the individual biomarker can be combined with a biomarker reference panel and the cervical cancer lesion can be classified according this comparison. Preferably, the biomarker reference panel of the method comprises a constituent panel developed using cervical cancer, high grade cervical lesion, low grade cervical lesion, and control group populations.

Moreover, the invention provides a method of treating cervical cancer or preventing the onset of cervical cancer and reducing the extent to which it occurs. Such method comprises administering to the female a therapeutically effective amount of an agent which targets and blocks or decreases the function of one or more of the biomarkers. In one case, the agent blocks interaction between Myc and HPV E6. In other cases, the agent blocks or reduces the expression level of hTERT, IGFBP-3, transferrin receptor, beta-catenin, HPV E6, or HPV E7. In a particular case, the agent blocks signaling through the beta-catenin pathway. Exemplary therapeutic agents in such methods include, but are not limited to, small molecules, polypeptides, antibodies, and nucleic acids. In specific embodiments, the present invention contemplates the use of antisense nucleic acids or RNA interference (RNAi) nucleic acids to block or reduce gene expression of one or more of the above biomarkers.

The methods can be used alone or in combination with other anti-viral or anti-cancer therapeutic approaches (e.g., administration of an anti-viral or anti-cancer agent, radiation therapy, phototherapy or immunotherapy) directed to treatment or prevention of cervical cancer or virus infections. Thus, the methods of the invention may further include as optional ingredients one or more agents already known for their use in the inhibition of cervical cancer, for added clinical efficacy. These agents include interleukin-2,5′-fluorouracil, nedaplatin, methotrexate, vinblastine, doxorubicin, carboplatin, paclitaxel (Taxol), cisplatin, 13-cis retinoic acid, pyrazoloacridine, vinorelbine, artemisinin, and artemisinin analogs. Appropriate amounts in each case will vary with the particular agent, and will be either readily known to those skilled in the art or readily determinable by routine experimentation. In other cases, the subject methods of the invention may further include as optional ingredients one or more agents already known for their anti-viral effects, including 5′-fluorouracil, interferon alpha, imiquimod, lamivudine, arsenic trioxide, capsaicin, nucleoside analogues (e.g., acyclovir), and antiviral vaccines [63].

US20080187513 - Treatment of Solid Cancers

US20080187513 patent contemplates a method for treating or preventing cancer growth and metastasis by administering angeloyl substituted ingenanes or derivatives directly or proximally to the tumor, in order to induce primary necrosis in the cancer cells and to stimulate the generation of cancer-specific T-cells. The cancer-specific T-cells include $CD8^+$ T-cells and $CD4^+$ T-cells or their precursors. The angeloyl substituted ingenanes can be either

combined with genetic, immunological or cytological agents which enhance, co-operate or otherwise synergize the induced cancer-specific T-cells, or with other anti-cancer regimens including radiotherapy and chemotherapy. Angeloyl substituted ingenanes can be co-administered with a cancer vaccine such as a dendritic cell vaccine or a vaccine based on virus vector or recombinant protein or cancer cell lysate, which is capable of presenting a cancer antigen or epitope to the immune system.

The method of the present invention assists in the treatment of primary tumors and prevents or reduces the growth of secondary tumors. Thus, this immunostimulatory chemoablation therapy not only debulks the tumor burden but in so doing also induces cancer-specific T-cells such as $CD8^+$ T-cells and $CD4^+$ T-cells. The angeloyl substituted ingenanes or derivatives may be synthetically produced or may be derived from extracts of a plant of the Euphorbiaceae family, particularly *Euphorbia peplus* [64].

US20080286781 - Compositions, Kits, and Methods for Identification, Assessment, Prevention, and Therapy of Cervical Cancer

In US20080286781 the invention relates to cancer markers that can be exploited for cervical cancer diagnosis, staging, prognosis and treatment, including carcinomas (carcinoma *in situ*, invasive carcinoma, metastatic carcinoma) and pre-malignant conditions (dysplasia, including CIN or SIL). The invention provides a diagnostic method of assessing whether a patient has cervical cancer or has higher than normal risk for its development, by comparing the level of expression of a marker of the invention in a patient sample and the normal level of expression of the marker in a control, e.g., a sample from a patient without cervical cancer. The markers are selected such that the positive predictive value is at least about 10%, preferably about 25%, more preferably about 50% and most preferably about 90%. The methods of the invention may be used to measure response to therapy, for example, to verify the reduction in tumor burden, evaluating the expression of the marker in a patient before, during and after treatment.

The invention further provides a method of inhibiting cervical cancer in a patient, which consists in obtaining a sample comprising cancer cells from the patient, separately maintaining aliquots of the sample in the presence of a plurality of compositions, comparing expression of a marker of the invention in each of the aliquots and administering to the patient at least one of the compositions which significantly lowers the level of expression of the marker The cells may be found in a cervical smear or body fluid ncluding blood, lymph, ascitic fluids, gynecological fluids, urine, and fluids collected by vaginal rinsing.

Additionally, the invention includes the use of untibodies which bind specifically with a marker protein or a fragment of the protein. The invention also provides methods for making such antibody, antibody derivative, and antibody fragment, which may comprise immunizing a mammal with a protein or peptide containing the entirety of a marker protein, wherein the protein or peptide may be obtained from a cell or by chemical synthesis. The methods of the invention also encompass producing monoclonal and single-chain antibodies, which would further comprise isolating splenocytes from the immunized mammal, fusing the isolated splenocytes with an immortalized cell line to form hybridomas, and screening individual hybridomas for those that produce an antibody that binds specifically with a marker protein or a fragment of the protein [65].

US20080213220 - Cancer-Targeted Viral Vectors

US20080213220 patent relates to viral vectors that are targeted to cancer cells, including cervical cancer. The viral vectors of the invention are adenoviruses having a PEG-3 promoter driving the expression of the viral genes E1A and E1B. The PEG-3 promoter exhibits increased activity in malignant cells. Adenoviruses of the invention show increased replication in malignant cells, thereby producing a cytopathic effect. The viral vectors of the invention further comprise additional genes of interest, and may have altered capsid proteins that may enhance infection of and target infection to cancer cells. Additional cell types derived from diseased states in which the PEG-3 promoter is selectively active are also therapeutic target of the viral vectors of the instant invention including those generating allergic, autoimmune and inflammatory responses [66].

WO2008104804 - Proteins

WO2008104804 patent relates to the identification of membrane proteins associated with cervical cancer, among other malignancies. These proteins can be useful as tumor markers and as targets against which antibodies or other pharmaceutical agents can be made. The first aspect of the invention provides methods of treating cervical cancer that consists in administering to a cervical cancer patient a therapeutically effective amount of a compound that modulates (up regulates or down regulates) or complements the expression or the biological activity of one or more proteins of the invention.

The second aspect of the invention consists on a method for cervical cancer detection, diagnosis and/or screening and disease progression monitoring. It comprises detecting the presence or level of the proteins of the invention, and also the fragments or nucleic acid that encodes these proteins. This may include the step of obtaining a biological sample (serum or tissue) from the patient. The analyses can be made either through imaging technologies or through immunohistochemistry on tissue sections. Immunohistochemistry is a technique which detects the localization of an antigen by the use of specific labeled antibodies. Antigen-antibody interactions can be visualized by a marker such as fluorescent dye, enzyme, radioactive element or colloidal gold.

In a further aspect, this invention provides the use of the proteins as vaccine compositions, for either prophylactic or therapeutic purpose. The vaccine compositions can include one or more immuno stimulants [67].

US20080260729 - Method of Treating Cancer Comprising a VEGF-B Agonist

US20080260729 patent provides a method of inhibiting the growth of cancer including tumor tissue and precancerous tissue using an antagonist of VEGF-B. Com-

positions are also provided comprising one or more VEGF-B antagonists alone or in combination with other anti-cancer agents or other angiogenesis inhibiting agents. An antagonist contemplated by the present invention may be an antibody which inhibits interaction between VEGF-B and VEGFR-1, an antisense compound which reduces VEGF-B expression, or an interfering nucleic acid which reduces VEGF-B expression. The preferred antibodies bind to VEGF-B and interfere with VEGF-B interaction with its receptor. The antibody and other antagonists are proposed for use in treating certain conditions mediated in whole or in part, or directly or indirectly, by VEGF-B. Preferably, the antibodies are monoclonal antibodies or antigen-binding fragments thereof. Even more preferably, the antibodies are humanized antibodies including deimmunized or chimeric antibodies or human antibodies suitable for administration to humans. Antibodies in accordance with this invention include the murine monoclonal antibodies 1C6, 2F5, 2H10 and 4E12, and humanized, deimmunized or chimeric forms of mAbs 1C6, 2F5, 2H10 and 4E12 [68].

US20070269407 - Use of Interleukin-19 to Treat Cervical Cancer

US20070269407 patent is based on treating a mammalian having HPV infection, cervical dysplasia, cervical intra-epithelial neoplasia and carcinoma of the cervix with interleukin-19 (IL-19). The invention also provides a method for inhibiting the growth of cervical cancer cells by bringing IL-19 or fragments comprising helices A-D of IL-19, into contact with these cells. The quantities of IL-19 for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medications administered. Methods for administration include intravenous, peritoneal, intramuscular, transdermal or administration into the lung or trachea in spray form by means or a nebulizer or atomizer. Dosage ranges would ordinarily be expected from 1μg to 1000μg per kilogram of body weight per day. However, the doses may be higher or lower as can be determined by a medical doctor with ordinary skill in the art.

For cervical cancer diagnosis, suitable detectable molecules (radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles) may be directly or indirectly attached to IL-19. For treatment purposes, IL-19 can be administered in conjunction to radiation and chemotherapic agents, such as bleomycin, chlorambucil, epirubicin, 5-fluorouracil, ifosfamide, mitomycin, methotrexate, vincristine, cisplatin and vinblastine. Cytotoxic molecules may be directly or indirectly attached to IL-19, and include bacterial or plant toxins, as well as therapeutic radionuclides, such as iodine-131, rhenium-188 or yttrium-90. In addition, IL-19 polypeptide-toxin fusion proteins can be used for targeted cell or tissue inhibition or ablation. IL-19 cytokine fusion proteins can be used for *in vivo* killing of cervical cancer, where IL-19 receptors are expressed. The described fusion proteins enable targeting of a cytokine to a desired site of action, thereby providing an elevated local concentration of cytokine. IL-19 polypeptides target an undesirable cancerous cell or tissue, and the fused cytokine mediated improves target cell lysis by effector cells. Suitable cytokines for this purpose include interleukin 2 and granulocyte-macrophage colony-stimulating factor [69].

US20070166328 - Genetic Immunization Against Cervical Carcinoma

US20070166328 patent is an alphavirus vector system comprising nucleic acid derived from a HPV. Alphariuses include a nucleocapsid with one copy of a single-stranded RNA molecule surrounded by envelope containing spike proteins. Alphavirus RNA has a positive polarity, thus enabling the genomic RNA to initiate an infection when introduced into the cytoplasm of a cell. Further, the RNA is self-replicating since it encodes its own replicase, wherein replication results in high-level expression of the viral proteins in host cells.

The nucleic acids are derived from a HPV type 16 or type 18, and can be a gene, a functional part of a gene, a precursor of a gene, a transcribed gene on any nucleic acid level or a gene product derived therefrom that can overcome cell cycle suppression. The cell cycle suppression may be overcome by inactivating major tumor suppressor proteins, such as P53 and pRB gene products, respectively, leading to loss of normal cellular differentiation and the development of a carcinoma.

The alphavirus viral system is suited to safely induce cellular immune responses against oncoproteins such as HPV 16/18 E6 and E7. The invention further discloses an alphavirus vector system or other viral vector systems wherein the nucleic acid further encodes a cytokine gene or functional fragment thereof. Cytokines are primarily involved in signaling between cells of the immune system. It is provided to use Granulocyte-Macrophage Colony-Stimulating-Factor (GM-CSF) and/or Interleukin 12 (IL-12). However, the cytokines IL-2, IL-6, IL-18, and others are also contemplated.

The invention also provides the incorporation of an alphavirus vector system and/or a cell infected with an alphavirus vector system for the preparation of a vaccine for cervical cancer treatment. The method includes providing an alphaviral vector system with a broad host range comprising nucleic acid encoding tumor antigens devoid of capacity to bind to the cellular tumor suppressor products pRB and P53 and capable of inducing an HPV-specific cytotoxic T lymphocyte (CTL) response against HPV-transformed tumor cells expressing tumor antigens. CTLs can destroy cells expressing foreign antigens through recognition of foreign peptides generated within the cell, transported to the cell surface and presented by histocompatibility complex (MHC) class I antigens. The CTLs are, thus, potentially powerful agents of tumor cell destruction [70].

US20060029613 - Biological Compositions and Methods for Treatment of Cervical Cancer

US20060029613 patent relates to biological or oral compositions useful for subjects with cervical cancer. The methods of the invention comprise culturing yeast cells in the presence of a series of electromagnetic fields, such that the yeast cells become metabolically active. The electromagnetic fields used are each defined by one of five

frequency ranges and a broad range of field strength. The starting yeast cells are commercially available and accessible to the public as *Saccharomyces.* The methods for making the biological compositions of the invention further comprise conditioning the activated yeast cells in plant extracts and the gastric juice of animals, while in the presence of another series of electromagnetic fields.

The methods of manufacturing also comprise expanding the number of activated or activated and conditioned yeast cells in large scale cultures in the presence of yet another series of electromagnetic fields, performing quality control measures, and packaging. Pharmaceutical compositions of the invention comprise activated and conditioned yeast cells and one or more pharmaceutically acceptable excipients or carriers. Additional ingredients, such as vitamins and/or flavors may be added to the biological compositions to form the oral compositions of the invention. Such additional carriers and ingredients can improve the healthful benefits, pharmacological properties, and organoleptic characteristics of the oral compositions. During the manufacturing process, the activated or activated and conditioned yeast cells may be dried and stored for a period of time. The biological or oral compositions of the invention are ingested by the subject or used as an additive to be incorporated into food to be consumed by the subject. Dietary supplement and nutritional compositions comprising activated and conditioned yeast cells are encompassed by the invention.

The biological composition of the invention can retard the growth of cervical cancer cells and prolong the time of survival of an animal with cervical cancer which received the composition orally [71].

US20060039919 - Fusion Protein for Inhibiting Cervical Cancer

US20060039919 patent refers to a fusion protein for inducing immune response in cervical cancers. The fusion protein of the present invention can effectively inhibit the proliferation of carcinoma cells, induce cytotoxic T lymphocytes (CTL) and antibody protection *in vivo* and destroy the infected cells by presenting the antigen. The pharmaceutical composition of the present invention also comprises a medical compound such as a fusion protein for preventing or inhibiting cancer induced by HPV type 16, wherein the compound is able to control the proliferation or the increase of carcinoma cells. The invention also discloses an antibody composition, which targets the antigen of E7 peptide *in vivo* [72].

US6825226 - Apoptosis Inducing Adamanthyl Derivatives and their Usage as Anti-Cancer Agents, Especially for Cervical Cancers and Dysplasias

US6825226 patent relates to the discovery that specific adamantyl or adamantyl group derivatives containing retinoid-related compounds induce apoptosis of cancer cells and therefore may be used for the treatment of cancer, including advanced malignancies. Also, the present invention relates to novel adamantyl or adamantyl group derivatives containing retinoid compounds and their usage for prevention of cancer, keratinization disorders and dermatological conditions. More specifically, it has been shown that such adamantyl compounds, e.g., 6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoicacid, 2-[3-(1-adamantyl)-4-methoxyphenyl]-5-benzimidazole carboxylic acid, and 6-[3-(1-adamantyl)-4,5-methylenedioxyphenyl]-2-naphthoic acid, can be used to treat or prevent cervical cancers and precancers such as cervical dysplasias, including high grade and low grade dysplasias [73].

US20030161811 - Method for Treating Cervical Cancer

Molecular and epidemiologic studies have demonstrated a strong relationship between HPV, cervical intraepithelial neoplasia, (CIN), and invasive carcinoma of the cervix. This invention refers to administering interleukin-20 (IL-20) to a mammalian having cervical cancer or HPV infection. The invention also provides a method for inhibiting the growth of cervical cancer cells by bringing IL-20 into contact with these cells. Interleukin-20 (formally called Zcyto10) can be produced according to the method described in International Patent Application US98/25228 filed on Nov. 25, 1998. The human IL-20 polypeptide is comprised of a sequence of 176 amino acids.

IL-20 can be administered intralesionally, or intramuscularly for localized disease. For metastatic disease, IL-20 can also be administered by intraperitoneal administration including intravenous administration. IL-20 can be administered alone or in conjunction with standard therapies such as surgery, radiation or other chemotherapeutic agents such as bleomycin, chlorambucil, epirubicin, 5-fluorouracil, ifosfamide, mitomycin, methotrexate, vincristine, cisplatin and vinblastine.

Cells infected with HPV can be treated with IL-20 to inhibit the proliferation of the virus. Anogenital warts caused by HPV type 6, 11, 16, 18, 31, 33 and 35 are transmitted sexually and have an incubation period of 1 to 6 months. Endocervical wart infections caused by type 16 or 18 have been implicated as a cause of cervical intraepithelial neoplasia and cervical cancer. HPV types 16 and 18 generally do no not cause external genital warts, which are usually caused by types 6 and 1. IL-20 can be administered directly into lesions containing cells infected with HPV alone or with standard therapies such as interferon alpha or interferon beta both of which are commercially available. IL-20 can also be administered with other standard therapies for treating HPV including antimitotics such as podophyllotoxin, podophyllin, or 5-fluorouracil; caustics such as trichloroacetic acid; or interferon inducers such as imiquimod. The quantities of IL-20 for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medications administered [74].

The above described patents are summarized in Table **2**.

CURRENT & FUTURE DEVELOPMENTS

Despite significant advances in the understanding and management of cervical cancer, the mortality rates for this malignancy remain exceedingly high. It is believed that the greatest impact in cervical cancer burden will be achieved by primary prevention, either through better screening programmes or by restricting the spread of its viral cause.

Table 2. Overview of New Patents for Cervical Cancer Treatment

Patent Number	Invention	Mechanism of Action	Reference
US20080171051	c-FLIP inhibitor	Enhances cytotoxicity of CT	[62]
US20080113340	Biomarkers	Target therapy	[63]
US20080187513	Angeloyl substituted ingenanes	Induce primary necrosis; activate the immune system	[64]
US20080286781	Biomarkers	Target therapy	[65]
US20080213220	Viral vectors	Target cancer cells	[66]
WO2008104804	Biomarkers	Target therapy	[67]
US20080260729	VEGF-B antagonist	Target therapy	[68]
US20070269407	Interleukin-19	Activate the immune system	[69]
US20070166328	Alphavirus vectors	Activate the immune system	[70]
US20060029613	Metabolically active yeast cells	Retard tumor growth; prolong survival	[71]
US20060039919	Fusion protein	Activate the immune system	[72]
US20046825226	Adamantly derivates	Induce apoptosis of cancer cells	[73]
US20030161811	IL-20	Activate the immune system	[74]

CT: chemotherapy.

There is hope that the widespread HPV vaccination might benefit many women in the future, although these results are not expected for the next decades. Meanwhile, the main challenge is to improve the current therapeutic options, especially in respect to advanced lesions. Although no agent has shown superiority to cisplatin, much effort is being concentrated in the search for new drugs and optimal regimens to combine with radiotherapy. Additionally, various promising biologic agents are being developed and tested, and their results are enthusiastically awaited.

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CONFLICT OF INTEREST

Dr Daniela B. Cornelio, Rafael Roesler and Gilberto Schwartsmann do not have any financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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