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Enhancement of Conventional and Photodynamic Therapy for Treatment of Cervical Cancer with Cannabidiol

Radmila Razlog, MTech[1], Cherie Ann Kruger, DTech[2], and Heidi Abrahamse, PhD[2]

Abstract

Cervical cancer (CC) is the fourth most diagnosed cancer in women worldwide. Conventional treatments include surgery, chemo- and radiotherapy, however these are invasive and may cause severe side effects. Furthermore, approximately 70% of late-stage CC patients experience metastasis, due to treatment resistance and limitations. Thus, there is a dire need to investigate alternative therapeutic combination therapies. Photodynamic therapy (PDT) is an alternative CC treatment modality that has been clinically proven to treat primary CC, as well as to limit secondary metastasis. Since PDT is a non-invasive localized treatment, with fewer side effects and lessened resistance to dose repeats, it is considered far more advantageous. However, more clinical trials are required to refine its delivery and dosing, as well as improve its ability to activate specific immune responses to eradicate secondary CC spread. Cannabidiol (CBD) isolates have been shown to exert *in vitro* CC anticancer effects, causing apoptosis post treatment, as well as inducing specific immune responses, which obstruct tumor invasion and angiogenesis, and so hinder CC metastatic spread. This review paper discusses the current conventional and alternative PDT treatment modalities for CC, as well as their limitations over the last 10 years. It has a particular focus on the combinative administration of CBD with these treatments in order to prevent CC secondary migration and so possibly encourage future research studies to focus on this synergistic effect to eradicate CC.

Keywords

cannabis, cannabidiol, photodynamic therapy, cancer treatment, cervical cancer

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Introduction

Cervical cancer (CC) is the fourth most commonly diagnosed cancer in women worldwide.[1] Conventional CC therapies such as surgical excision, chemo- and radiotherapy are invasive and cause unwanted side effects.[2] Additionally, despite ongoing advances roughly 70% of late-stage CC patients experience metastasis due to entire surgical excision limitations and cervical cancer stem cell (CCSC) resistance to repeated therapies.[3,4] Therefore, alternative therapeutic combinations require investigation.

PDT is an alternative therapeutic modality, which has presented clinical evidence of CC primary eradication, as well as the ability to limit CCSC secondary metastasis.[5] Furthermore, PDT is known to be a very specific non-invasive localized treatment, with fewer side effects, shorter healing times and reduced resistance to repeated dose treatments, when compared to conventional treatments.[6] Current ongoing clinical trials have reported that PDT therapy with 5-aminolevulinic acid (ALA) has emerged as an effective and tolerable treatment strategy for the control of CC.[7-10] Nevertheless, these clinical trials require improvement in relation to investigating enhanced PDT treatment to activate specific immune responses to fully eradicate CC secondary migration.[11]

[1]Department of Complementary Medicine, Faculty of Health Sciences, University of Johannesburg, Doornfontein, Johannesburg, South Africa
[2]Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, Doornfontein, Johannesburg, South Africa

Corresponding Author:
Cherie Ann Kruger, Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, P.O. Box 17011, Doornfontein, Johannesburg 2028, South Africa.
Email: cherier@uj.ac.za

Recently, studies by Nkune et al. (2021 and 2022) and Mokoena et al. (2019)[12-14] have published promising results showing that utilizing PDT as a primary treatment for colorectal and breast cancer in combination with CBD ensures complete primary tumor ablation, as well as activates specific immune responses to limit migratory secondary spread. According to *in vitro* CC treatment studies performed by Ramer et al. (2010 and 2008), Contassot et al. (2004) and Eichele et al. (2009), Lukhele and Motadi (2016),[15-19] it was noted that CBD is able to activate specific immune responses, which can aid in the treatment of the primary, as well as secondary treatment of CC, since it may mitigate its metastasis and secondary spread.

Thus, to promote the overall effectiveness of both conventional and alternative PDT CC treatment strategies, as well as overcome their own individual limitations, especially in relation to CC resistance mechanisms and its aggressive metastatic nature, there is a strong need to investigate combinative therapeutic approaches. This review paper focuses on current CC treatment modalities over the last 10 years, as well as investigates the possible utilization of synergistic CBD therapies in order to possibly encourage future research studies to investigate the comprehensive synergistic eradication of CC.

Cervical Cancer

Cervical cancer (CC) is a malignant form of tumor which originates in the cervix It is divided into 2 histological types, namely adenocarcinoma (AC) and squamous cell carcinoma (SCC).[20] SCC originates from the squamous cell outer lining of the cervix, whereas AC originates from inner glandular cervical cells.[20] SCC has a higher occurrence rate of about 70% when compared to AC.[20]

Worldwide, CC is commonly diagnosed in women especially within low- and middle-income countries such as South Africa, India, China, and Brazil.[1] In 2020, a worldwide total of 604 127 new cases of CC was reported with 341 831 of these cases resulting in death.[1] The vast majority of new cases (84%) and 90% of CC deaths occur in low- and middle-income countries, due to the high incidence rates of women being infected with the sexually transmitted human papilloma virus (HPV).[20] HPV persistent infection in women over the age of 35 years old is responsible for approximately 70% of all diagnosed CC cases in low- and middle-income countries worldwide.[20] Other factors which may also contribute to the overall incidence rate of CC include lack of health care access in relation to early diagnosis and efficient treatment regimes, public awareness, smoking, the use of oral contraceptives and HIV co-infections.[20] The common signs and symptoms of CC include unusual vaginal discharge, vaginal bleeding (especially after intercourse), pelvic discharge and pain after intercourse.[20]

An estimated 11 million women globally will be diagnosed with CC in the next 10 to 20 years, so research and development into early detection and diagnosis, as well as treatments requires further investigation.[20]

Stages of Cervical Cancer Stages

There are 4 stages of CC: in stage I the cancer is isolated to the cervix region only; in stage II the cancer has spread to the upper two-thirds of the vagina or into the tissue surrounding the uterus; in stage III the cancer has spread to the lower third of the vagina and/or pelvic wall, and/or has caused kidney problems, and/or has shown lymphatic spread; in stage IV the cancer has spread beyond the pelvis, or has spread to the bladder lining, or rectum or other parts of the body.[21]

Conventional Treatments for Cervical Cancer

Currently, there are 5 standard conventional types of treatment available for CC, which include surgery, radiation therapy, chemotherapy, targeted therapy, and immunotherapy.

The type of treatment applied individually or in combination depends on the stage of CC at time of diagnosis.[22] For stage I CC, surgery such as conization or total/modified hysterectomy with internal radiation therapy is used.[22] Within stage II CC, combinative radiation and chemotherapy following radical hysterectomy and removal of pelvic lymph nodes is often considered.[22] For stage III CC combinative radiation and chemotherapy, followed by internal radiation therapy to shrink the tumor before full surgical hysterectomy and removal of pelvic lymph nodes, with follow-up chemotherapy often applied.[22] Within stage IV CC chemotherapy and radiation therapy can be administered as palliative care to relive cancer symptoms, as well as for comfort.[22] Other possible treatment options for stage IV CC however can include drastic surgical pelvic exenteration or clinical trials of targeted immunotherapies.[22]

Even though these conventional treatment modalities have shown promise, the unwanted short and long terms side effects are vast.[21] Surgery at any stage of CC is highly invasive and painful.[21] Radiation therapy is known to induce unwanted DNA damage in normal healthy cells, leading to loss in cellular recovery, cell cycle arrest and unrepairable damage, whereas chemotherapy is toxic to healthy tissues and so brings about short-term side effects such as hair loss, vomiting, diarrhea, coughing, swelling of the legs and weight loss.[23] The long term side effects from either radiation or chemotherapy include permanent abdomen, back or leg pain, trouble urinating, and feeling tired.[23] Moreover, additional treatment options such as targeted immunotherapies are novel and so remain in clinical stage trials, whereby their overall effectiveness remains unknown.[23]

Since the early detection of CC remains very poor due to lack of education and health care (especially in developing countries), as well as patients being asymptomatic and lack of accuracy in diagnostic pap smears, CC in women often goes by undiagnosed until its late stages, when patients start experiencing symptoms such as abdominal pain or unexplained vaginal bleeding.[24] Generally, 44% of CC cases are diagnosed in stage II and 38% in either stage III or IV.[24] Thus, advanced CC is one of the major leading cancer related mortalities in low- and medium-income countries mostly due to poor early screening, as well as lack of effective treatment regimens caused by therapy resistance and recurence.[24]

According to the Federation of Gynaecology and Obstetrics (FIGO) stage II CC has a 46% chance and stage III to IV has a 70% chance of recurrence, so patients' full recovery rates from CC remain very low.[3] Furthermore, recurring CC treatment remains a constant challenge and a patient's prognosis is often poor with an overall survival rate of less than 5% despite following intensive chemo- and radiotherapy treatment regimens.[3] This poor prognosis of recurrent CC is usually attributed to numerous factors including CC tumor stem cell biological behavior, limitations of surgical complete excision, as well as resistance to repeated radio- and chemotherapy.[3] Thus, due to the complex characteristics and high risk of CC recurrence, conventional treatment selections often have to consider the staging of prognosis, as well as the advantages/limitations of each therapy.[24]

Cervical Cancer Conventional Treatment Resistance

Cervical cancer stem cells (CCSCs) have been identified as the underlying cause of relapse and resistance to repeated chemo- and radiotherapy after successful primary conventional treatments have been applied.[4]

Various CCSC cellular and molecular mechanisms, as well as genetic mutations allow for excessive anticancer drug efflux to occur, and decreased drug accumulation contributes to its treatment resistance.[4] CC anti-drug resistance can be divided into inherent and acquired resistance.[4] Within the inherent form, anti-drug resistance exists before cancer exposure to treatment drugs.[4] This type of resistance is apparent in CCSC genes which control tumor cell proliferation and apoptosis.[4] Whereas acquired resistance develops after the first treatment with anticancer drugs, due to altered target anticancer drug level expressions.[4] Recently, CCSC microRNAs (miRNAs) such as long noncoding RNAs and p53 mutated transcription factor, which are involved in vital cell cycle biological processes have been identified as the major role players in CC malignancy development and anticancer drug resistance.[4,25] Yang et al. (2020)[2] state that the main reasons for the failure of conventional CC anticancer drug treatments are metastasis, recurrence, heterogeneity, resistance to chemotherapy and radiotherapy, and the avoidance of immunological surveillance which CCSCs cause. This study went on to state that since CCSCs are able to self-renew with differentiation potential (due to P53 mutations), as well as arrest the G0 cell death phase cycle, they are able give rise to new drug resistant tumors which confer aggressive secondary spread and metastasis.[2] The arrest of the G0 cell death phase cycle is due to CCSCs abilities to hinder the cellular signaling of the Phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway, as well as downregulate normal P53 expression.[2] This ability allows CCSCs tumor cells to undergo uncontrolled cellular proliferation and develop highly metastatic tumors, which are anti-apoptotic, and so extremely resistant to various conventional treatments.[2]

Alternative Photodynamic Therapy for Cervical Cancer Treatment

In recent years research has begun to focus on alternative methods that are able to effectively treat CC, as well as to reduce conventional treatment invasiveness, unwanted side effects, rate of recurrence and metastasis.[5]

Photodynamic therapy (PDT) is an alternative treatment modality that has been proven to treat primary CC, as well as eradicate CCSCs to prevent secondary metastasis.[5] PDT has numerous advantages over conventional therapies, since it is a very specific non-invasive localized treatment, with very few side effects, it has a short healing time with no scarring, and it is highly tolerant to repeated doses with little to no resistance.[6] Furthermore, PDT may be considered for patients as an alternative treatment, as it allows them to preserve their fertility, whereas surgery, chemo- and radiotherapy often induce sterility in patients.[5]

PDT Mechanism of Action for Cervical Cancer Treatment

PDT treatments utilize a light-excitable dye known as a photosensitizer (PS) which is administered to a patient (Figure 1).[26] The PS is given time to distribute throughout the patient's body. Due to the enhanced permeability and retention effect (EPR) which cancer cells possess, a PS is able to accumulate in tumor cells passively and selectively.[9] Once PS selective accumulation has occurred an external light source at particular wavelength of irradiation is applied to the localized tumor in the patient's cervical area via hysteroscopy.[9] The application of red laser light excites the localized tumor PS from its single ground state to an excited triplet state.[26] In a type I reaction the PS in its excited triplet state reacts with the tumor cells' surrounding

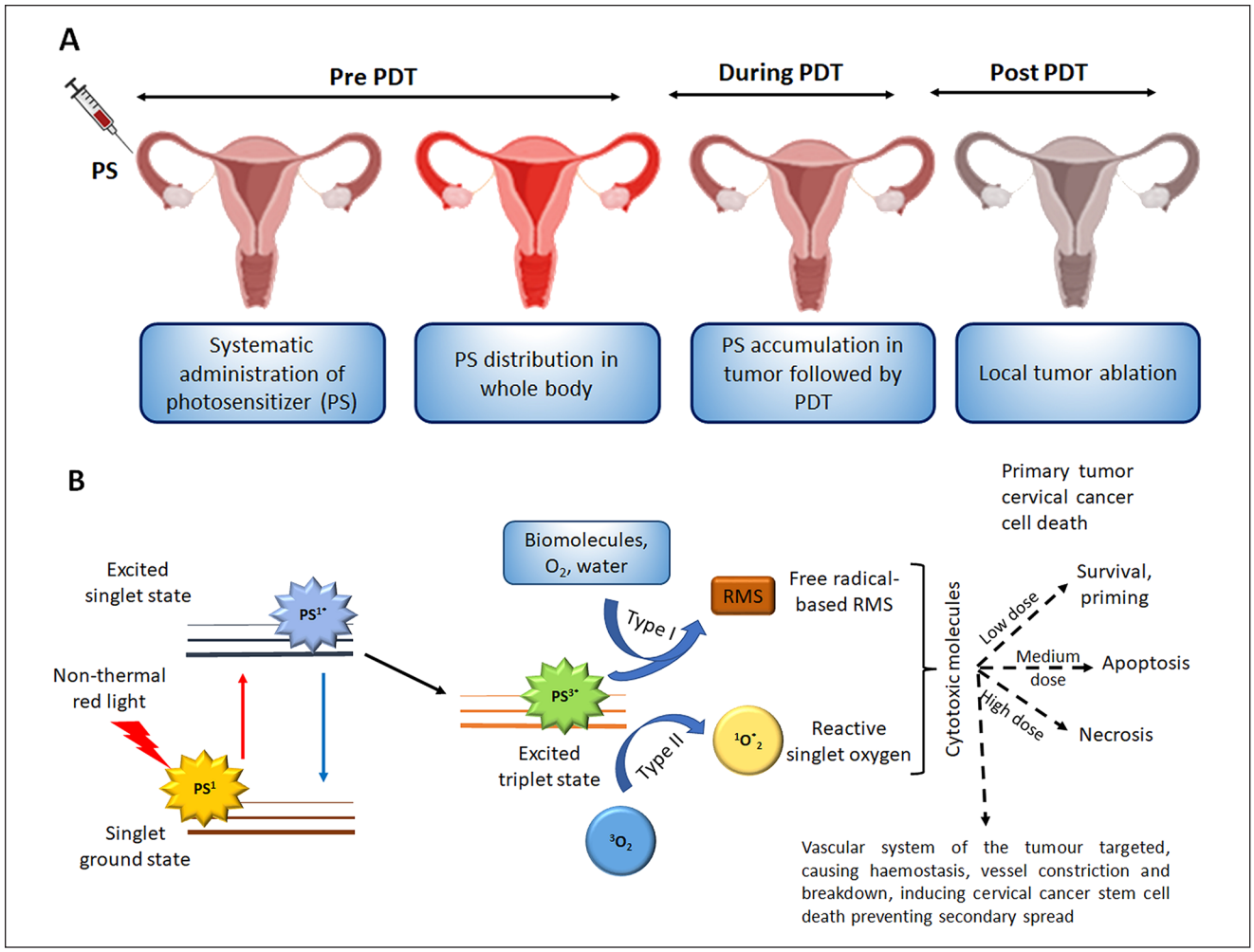

Figure 1. Photochemical and photobiological overview of CC PDT. (A) Clinical process of PDT and (B) Schematic representation of the Jablonski diagram showing PDT mechanism of action for tumor destruction.

biomolecules, oxygen, and water to produce free radical molecular species (RMS), such as reactive oxygen species (ROS).[26] In type II reactions the excited triplet state PS combines with triplet state oxygen ($^3O_2$) to produce reactive singlet oxygen ($^1O_2$).[26]

The generation of cytotoxic ROS and $^1O_2$ free radical species induces oxidative stress in primary and secondary CC tumor cells, which in turn causes cell death by either apoptosis or necrosis.[26] These forms of cell death induced by oxidative stress in tumor cells cause destruction to various internal biomolecules such as DNA, proteins, and ligands from which a primary CC tumor cannot recover and is destroyed.[26]

Additionally, this form of PDT treatment is also able to activate various other antitumor immune responses, which in turn cause damage to a tumors vascular nature and so further enhances CCSC eradication to prevent secondary spread.[5] When PDT targets the vascular nature of a tumor, it causes hemostasis, vessel constriction, and breakdown, which leads to depletion of oxygen and nutrients to a tumor and so assists in primary, as well as secondary CCSC destruction.[5] Thus, PDT CC treatment is able to force localized tumor tissue demolition, as well as provoke substantial anti-tumor responses and acute inflammatory process, all of which contribute to the eradication of primary CC and aid in preventing its secondary spread.[5]

Recent PDT Clinical Studies for Cervical Cancer Treatment

A systematic review study by Zhang et al. (2018)[27] was conducted in 2018 in which168 randomized clinical trials of CC PDT treatment were identified. It was reported that PDT significantly increased the remission rate of patients by 82%, however it went on to note that future clinical studies were necessary to identify the most effective and least toxic PS

that doesn't compromise safety. One of the most prominent studies noted within this review was by Park et al. (2016), who reported the successful treatment of 50 early-stage CC patients with Photofrin® PS PDT with a 95% recovery, although unwanted photosensitivity and inflammation were experienced by patients. The researchers further suggested that combined chemo-PDT was essential in clinical cases whereby the CC had metastasized.[27,28] However, studies by Inada et al. (2019) investigated the effective FDA approved pro-drug hexaminolevulinate (HAL) PS PDT treatment in 56 CC patients and 90% of the patients noted a full response to the treatment with no recurrence, progression, and/or lesions 2 years post treatment, with limited side effects.[29] More recently, a study conducted by Ivanova et al. (2020), reported the successful Photoditazine® and Photolon™ PS PDT treatment of CC in 45 patients, whereby 86% of the patients had no reccurrence 5 years post treatment.[6] However, the most notable and recent preclinical and clinical CC PDT phase treatment trials were conducted by Gunaydin et al. (2021) and Li et al. (2020).[7,8] These studies investigated the use of FDA approved ALA PS-based PDT treatment in 77 CC patients and noted low morbidity, with limited side effects and a 94.81% remission rate 1 year post treatment.[7,8] In addition, studies by Li et al. (2020), Afanasiev et al. (2021), and van Straten et al. (2017) reported that CC ALA PS PDT within clinical phase trials has emerged as the most effective and harmless treatment strategy for the current control of CC; however it requires further investigation before this treatment can become available to the public health system, due to limitations of eradicating secondary spread.[8-10]

Limitations and Future of PDT for Cervical Cancer Treatment

Clinical and preclinical CC PDT studies have shown that low-dose PDT regimens can allow for CC tumor survival, as well as induce anti-tumor priming immunity, whereas medium dose PDT treatment can induce favorable apoptotic tumor cell death and high dose PDT can cause necrotic tumor ablation (Figure 1).[7,8] Thus, medium to high dose PDT is often required in CC treatment in order to achieve local primary tumor control, as well as ensure CCSC immune suppression of secondary spread.[7,8] Overall, accumulating evidence indicates that the efficacy of CC PDT relies on its capacity to influence tumor-host interaction, while tipping the balance toward the activation of specific immune responses and vascular shutdown to stop cancer metastasis.[11] Therefore, in relation to CC PDT being able to surpass clinical trial phases, strategies to ensure controlled high dosing, with improved light source delivery strategies to induce deep tissue phototoxicity and limited skin photosensitivity, as well as enhanced abilities to activate specific immune responses to be able to fully eradicate secondary spread, require further investigation.[11]

Even though the latest clinical trials have shown the enormous potential of CC PDT, other preclinical trials indicate that there is still room for improvement.[30] Newer combinative PDT treatment strategies, which require further investigation, have been suggested for complete CC tumor elimination.[30] Among some of the current combination studies, there has been suggestive evidence by Nkune et al. (2021 and 2022) and Mokoena et al. (2019) that when primary PDT treatment of colorectal and breast cancer was combined with cannabidiol (CBD), complete cancer primary cell cancer growth ablation and migratory suppression for secondary spread was possible.[12-14]

Cannabis and Cannabidiol

The ancient plant *Cannabis sativa* L. has been used as a medicinal phytotherapeutic remedy for centuries,[31] owing to its production of hundreds of different compounds, including an abundance of phytocannabinoids.[32] Analysis of the plant's secondary metabolites including phytocannabinoids, terpenoids, sterols, and flavonoids, form a baseline of reference values useful for research and clinical studies to understand the "entourage effect" of cannabis as a whole, and also to determine the therapeutic potential for each individual compound contained, by applying modern scientific methodologies.[33] The most studied phytocannabinoids are the psychoactive Δ9-tetrahydrocannabinol (THC) and the non-psychoactive cannabidiol (CBD).[34] CBD has attracted the interest of the scientific community mainly due to its antioxidant, anti-inflammatory, anticancer and analgesic properties.[34] Based on these properties, CBD represents a chemical structure of high potential for the treatment of cancer.[35]

Cannabidiol in Cancer Treatment

Numerous cell culture and animal studies have demonstrated the antitumor effects of CBD in various cancer types, noting that it can prevent proliferation, metastasis, angiogenesis, as well as exert pro-apoptotic cell death effects.[36] *In vitro* and *in vivo* cancer research has shown that CBD can effectively control tumor growth; however, the antitumor effects appear to be largely dependent on cancer type and drug dose/concentration.[36] An extensive review by Seltzer et al. (2020)[37] discusses the most recent findings that strongly support CBD as a promising cytotoxic cancer drug. Additionally, other research studies suggest encouraging results in the treatment of, but not limited to, glioma, breast, lung, colorectal, prostate, and cervical cancer types.[19,37] Furthermore, studies by Zhelyazkova et al. (2020) have reported that CBD has the ability to stimulate various

immune system responses which result in the signaling of anti-tumor signaling pathways, which are capable of controlling/limiting metastatic tumor growth.[38] Within a recent review performed by Luschnig and Schicho (2019), on the abilities of CBD to treat various gynecological diseases, they noted that CBD drastically reduced CC *in vitro* cells growth via apoptotic cell death mechanisms, while successfully generating anti-tumor immune responses post treatment, aiding in the control of CCSC metastatic tumor proliferation and secondary spread.[39] Moreover, within *in vitro* CC treatment studies performed by Ramer et al. (2010 and 2008) and Lukhele and Motadi (2016),[15,16,19] it was noted that CBD is able to activate specific immune responses, which can aid in the treatment the primary, as well as secondary treatment of CC.

Studies by Velasco et al. (2016),[40] noted that the application of combinational anticancer therapies is far more advantageous than single-agent strategies, as they can act synergistically to reduce tumor growth. Thus, for advanced types of CC which present primary, as well as secondary progression at various levels, combinative primary CC PDT treatment could be enhanced when synergistically combining it with CBD. This approach can possibly hinder secondary CCSC growth and spread, for overall improved treatment outcomes. A study done by Go et al. (2020)[41] also supports this statement, by noting that in order to ensure complete tumor eradication, more synergistic cancer therapies require investigation, since combinative treatments allow for more cell death pathways to be targeted to ensure primary, as well as secondary tumor destruction.

Thus, understanding how CBD is able to regulate essential cellular processes involved in tumorigenesis, such as progression through the cell cycle, cell proliferation and cell death, as well as the interactions within the immune system, is crucial for improving existing and developing new therapeutic approaches for cancer patients.[42] Though the cellular response to CBD is complex, certain themes have emerged to explain its anti-tumor effects, which are highly beneficial for cancer treatment.[37]

Cannabidiol Induced Cervical Cancer Cell Death. The endocannabinoid system (ECS) is an important neuromodulatory system that consists of receptors, endogenous ligands, and metabolizing enzymes, which all contribute toward the synthesis and degradation of the endocannabinoids.[43] Through direct and indirect actions, intrinsic endocannabinoids and plant-based phytocannabinoids modulate and influence a variety of physiological systems which are controlled by the ECS, including the development and progression of cancer.[42]

Since the ECS regulates almost all levels of female reproduction, starting with oocyte production right through to birthing, should it become disrupted this can lead to the development of various gynecological disorders such as fertility issues and various cancers.[39] Cannabinoids, such as CBD can act on the ECS as specific antagonists and so potentially can influence any ECS dysregulation, thereby representing a new therapeutic option for the treatment of various gynecological cancers, including CC.[17,39]

Within a review by Ayakannu et al. (2020) findings suggested that the ECS may be targeted to restrain the development and progression of gynecological cancers, since the ECS is implicated in cancer cell proliferation, angiogenesis, metastasis, and apoptosis.[44] The findings from this review and direct studies on CC other studies by Rammer & Hinz (2008), Contassot et al. (2004) and Eichele et al. (2009) suggests that an imbalance in endocannabinoid homeostasis may promote CC development, proliferation, and migration; therefore, the ECS is an attractive target for pharmacological intervention in the fight against CC.[16-18] These studies noted that since the ECS involves different signaling pathways, which include activities mediated via cannabinoid receptors 1 and 2 (CB1, CB2), transient receptor potential vanilloid (TRPV1) ion channels and G-protein-coupled receptors 55 (GPR55), these would be ideal therapeutic targets for the treatment of CC.[16-18,44]

Experimental CC studies by Contassot et al. (2004), have shown that responses to ECS ligands are mediated by CB endocannabinoids within *in vitro* cultured Caski, HeLa, and CC29 CC cell lines.[17] CB1 and CB2 receptors consist of 2 endogenous bioactive lipids, namely N-arachidonoylethanolamine (also known as anandamide, AEA) and 2-arachidonoylglycerol (2-AG), which in combination with enzymes degrade CBD, to assist in ECS regulation and so aid in tumor reduction.[45]

There is growing evidence that CBD inhibits CC tumor growth and metastasis following its binding to GPR namely GPR55, GPR18, or GPR119, which cause either CB1 or CB2 receptors to become activated and in turn initiate tumor-specific apoptotic cell death.[17,39,45] Elevated GPR55, GPR18, or GPR119 gene overexpression in cancer is associated with reduced disease and metastasis-free survival.[46,47]

Studies by Contassot et al. (2004) and Laezza et al. (2020), have noted that CB1 and CB2 are both overexpressed by many cancer types, including CC.[17,48] The overexpression of CB1 receptor has been confirmed in female reproductive systems.[39] While CB2 receptor overexpression is mainly located in immune system tissues (i.e. spleen, tonsils, thymus, and bone marrow), and in immune cells (i.e. B cells, natural killer cells, monocytes, neutrophils, CD8+ and CD4+ T cells).[39] These CB2 receptor immune cells are involved in macrophage activation, which in turn target mutated tumor cells in order to initiate their programmed cell death.[42] Therefore, the activation of CB1 and CB2 receptors by CBD in most cases is antitumorigenic, that is, it inhibits tumor cell proliferation, induces apoptosis *in vitro*, as well as blocks angiogenesis and tumor invasion/metastasis *in vivo* (Figure 2).[47,48] Studies by Contassot et al. (2004) and Eichele et al. (2009) reported that within *in vitro* cultured CC cell lines (Caski, HeLa, and CC29 CC)

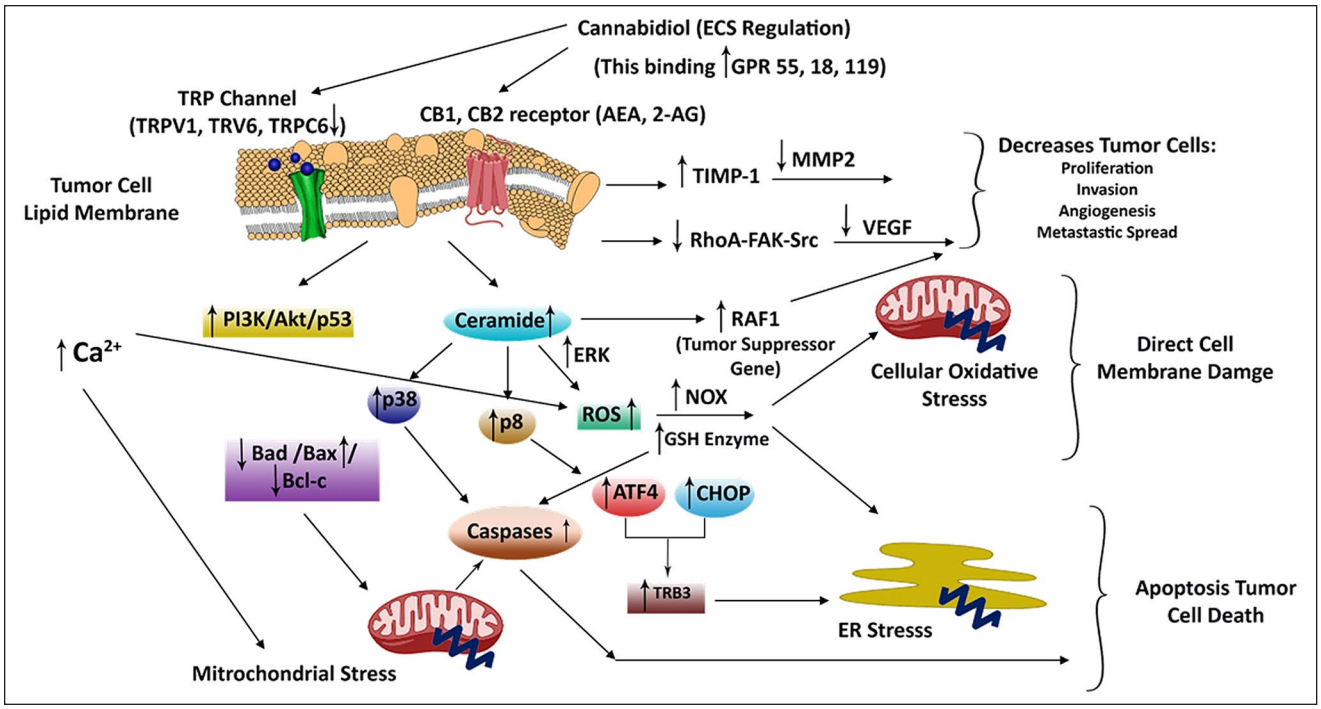

Figure 2. Schematic representation of cell signaling pathways associated with CBD induced CC cell death via ECS regulation.

treatment with the cannabinoid CBD had time- and concentration-dependent killing effects that were mediated by apoptosis, as well as decreased invasion.[17,18]

CBD Tumor Induced Cell Death Effect Through CB1 and CB2 Receptor Activation. Upon CBD binding to CB1 and CB2 receptors, various cellular signaling pathways become activated which inhibit tumor cell proliferation and invasion via apoptotic cell death induction (Figure 2).[47,49]

CB receptor activation over stimulates PI3K/Akt signaling pathways and cellular tumor antigen, p53 expression, which in turn causes an imbalance of Bad/Bax and Bcl-c cell cycle regulatory molecules.[46,49] This imbalance induces mitochondrial stress and caspase upregulation, resulting in apoptotic tumor cell death.[49] In humans p53 is a transcription factor for a number of target genes and is maintained constantly in the body to promote apoptotic cell death of abnormal cells.[25] However, in response to various external promoting cancer inducing stimuli (i.e. radiation, DNA damage, hypoxia, and UV light), p53 often mutates, lowering the expression of its normal apoptotic controlling form .[25] Overexpression of mutated p53 has been associated with more than 50% of all human cancers since it promotes tumorigenesis.[25] Bax and Bcl-2 are proteins which regulate apoptotic cell death via the mitochondria.[25] Within a normal cell death apoptotic process p53 proteins translocate to the cellular cytosol and trigger the oligomerization of Bcl-c protein with Bad, resulting in Bcl-c inhibition.[50] This in turn causes Bax protein to translocate to the mitochondria and release cytochrome-c, which results in apoptotic cell death.[25] However, when cancers show an overexpression of mutated p53 and an under-expression of normal p53, there is an imbalance between Bax and Bcl-2 protein expression.[50] This Bax and Bcl-2 protein imbalance has been linked to the development and progression of tumors, since they become resistant to undergoing normal apoptotic cell death induction so that their metastatic potential spread is inevitable.[50] Thus, cancer treatments need to focus on drugs which can effectively promote the expression of normal p53 gene pathways, in order to hinder cancer tumors anti-apoptotic abilities.[50]

Studies by Lukhele and Motadi (2016)[19] reported an *in vitro* CBD treatment study on an aggressive HeLa, as well as a metastatic ME-180 and a primary SiHa CC cell lines. All 3 types of CC cell lines noted high levels of mutated p53 overexpression.[19] Post CBD treatment all 3 cell lines noted a remarkably significant upregulation of normal p53 expression, with high expression levels of caspase 3 and Bax, as well as the down-modulated expression of Bcl-2.[19] These findings suggested that CBD promoted normal p53 pathways and so induced significant levels of apoptotic cell death in these types of CC cancer.[19]

Furthermore, studies have noted that the activation of CB receptors by the cannabinoid CBD increases the synthesis of ceramide, which is a pro-apoptotic sphingolipid that inhibits the *in vitro* and *in vivo* growth and survival of CC.[17,46] First, CB receptor activation initiates the intracellular accumulation of ceramide, which in turn activates

Raf1 tumor growth suppressor genes and stimulates extracellular signal-regulated kinases (ERK) cellular signaling pathways.[48] Sustained ERK signaling prompts cells to produce ROS, which in turn can cause direct tumor membrane damage and death.[48] The source of ROS production comes from the influx of Ca^{2+} via TRPV1 CBD downregulation, as well as the electron transport chain in the mitochondria and NADPH oxidase (NOX) transmembrane enzymes.[17,37] Endoplasmic reticulum (ER) stress and ROS production are directly related.[37] Each respective pathway can activate the other, with their actions culminating in the activation of mitochondria-stress mediated apoptotic cell death due to increased intracellular calcium Ca^{2+}.[37] According to Afrin et al. (2020),[46] CBD initiates ROS production to deplete the intracellular glutathione (GSH), via increased GSH-associated enzymes and so activates pro-apoptotic caspase proteins to induce tumor cell death. Furthermore, CBD promotes NADPH oxidase (NOX) transmembrane enzyme overexpression, which in turn is responsible for directing the rapid release of ROS, inducing cellular oxidative stress.[46] ROS production directly oxidizes nucleic acids, proteins, and lipids in tumor cells and so causes direct cell membrane damage.[37]

Secondly, the intracellular accumulation of ceramide causes the upregulation of stress-regulated proteins p38 and p8.[46] The upregulation of p38 causes an increase in caspases, which initiates downstream apoptotic tumor cell death.[48] The upregulation of p8 causes an increase in its downstream targets [cyclic AMP-dependent transcription factor (ATF4), C/EBP homologous protein (CHOP), and tribbles homolog 3 (TRB3)], which in turn induces ER stress driving phosphorylation and final apoptotic tumor cell death.[48] Studies by Ramer and colleagues (2010 and 2008)[15,16] reported that CBD treatment upregulated p38 expression within *in vitro* cultured CC HeLa and C33A cells and so initiated significant apoptotic tumor cell death. Additionally, studies by Lukhele and Motadi (2016),[19] agree with these findings noting that CBD activated p38 overexpression in HeLa, SiHa, and ME-180 *in vitro* cultured CC cells and so decreased tumor proliferation via apoptotic cell death pathways. Studies by Burstein et al. (2008),[51] further supports this finding by stating that acylamido analogs of endocannabinoids such as CBD selectively inhibit CC cancer cell proliferation via apoptosis.

CBD Effect on Non-CB1 and CB2 Receptors. Outside of CB1 and CB2 receptors, other non-CB receptors are also involved in the antitumor action of CBD and so represent another form of targeted cancer treatment (Figure 2).

Other families of ECS-related receptors include transient receptor potential (TRP) ion channels, which appear to be upregulated in many types of gynecological cancers.[39] Studies by Taylor et al. (2020) have reported that CBD doesn't only just bind to CB receptors, but also intracellularly binds to TRP ion channels, which are widely overexpressed in female reproductive tissues, and represses their expression.[45] TRP channels which promote cellular proliferation belong to 3 subgroups channels, namely TRPC (canonical), the TRPV (vanilloid), and TRPM (melastatin).[45] Studies by Lukhele and Motadi (2016) and Luschnig and Schicho (2019) have reported that TRPV1, TRPV6 and TRPC6 channel overexpression is related to the pro- carcinogenic effect of CC and that when CBD binds to these channels tumor metastatic growth can be hindered.[19,39] Studies by Laezza et al. (2020) suggest that the TRPV-dependent pro-apoptotic effect of CBD involves the influx of intracellular calcium (Ca^{2+}), which increases upon binding.[48] Increased Ca^{2+} levels in tumor cells triggers apoptosis via mitochondrial stress, as well as promotes ROS production and so inhibits cancer cell growth.[47] Additionally, studies by Contassot et al. (2004), Eichele et al. (2009) and Taylor et al. (2020), noted strong expressions of CB1 and CB2 receptors, as well as of TRPV1, in HeLa, CC29, Caski CC cell lines, and after CBD treatment significant pro-apoptotic effects were found.[17,18,45] Furthermore, studies by Ramer and colleagues (2010 and 2008) reported that CBD decreased the invasiveness of *in vitro* cultured CC HeLa and C33A cells in a dose-dependent manner, via TRPV1 downregulation.[15,16] Moreover, studies by Contassot et al. (2004) and Eichele et al. (2009) reported that in *in vitro* cultured CC cell lines (Caski, HeLa and CC29 CC) treated with the cannabinoid CBD had time- and concentration-dependent killing effects that were mediated by apoptosis, as well as decreased invasion, due to TRPV1 downregulation.[17,18]

CBD Inhibitory Effect on Tumor Migration, Invasion, and Angiogenesis. Various reports have highlighted that CBD can inhibit the spread of cancer by inhibiting various invasion, angiogenesis, metastasis, and migration mechanisms that tumors possess (Figure 2).[40,49]

First, when CBD binds to CB1 and CB2 receptors, the RHOA (ras homolog gene family, member A)- focal adhesion kinase—Proto-oncogene tyrosine-protein kinase Src (RhoA-FAK-Src) axis is inhibited.[52] This RhoA-FAK-Src inhibition initiates the downregulation of vascular endothelial growth factor (VEGF), which in turn halts tumor angiogenesis.[18,52]

Second, CBD CB receptor activation induces the release of tissue inhibitor matrix metalloproteinases-1 (TIMP-1), which serves as an endogenous inhibitor of matrix metalloproteinase 2 (MMP2) and so downstream blocks tumor angiogenesis and invasion.[48,52] Ramer and colleagues (2010 and 2008)[15,16] reported that CBD decreased the invasiveness of *in vitro* cultured CC HeLa and C33A cells in a dose-dependent manner, via the upregulation of TIMP-1 and downregulation of MMP. Since MMP2 serve as a group of enzymes which exert an imperative function during tumor

metastasis, when it is expression is downregulated by TIMP-1 CBD induced upregulation, a tumors invasion, and angiogenesis decrease.[15,16] Additionally, studies by Lukhele and Motadi (2016)[19] agree with these findings, noting that CBD downregulated TIMP-1 expression in HeLa, SiHa and ME-180 *in vitro* cultured CC cells and so decreased the invasiveness of CC.

Conclusion

Considering the key points drawn from this review, conventional CC therapies such as surgical excision, chemo- and radio- therapy are invasive and cause adverse side effects.[2] Furthermore, despite the ongoing advances which have been made in conventional CC therapies, roughly 70% of late-stage patients experience recurrence or metastasis due to the limitations of surgical excision and CCSC resistance to repeated radio- and chemotherapy.[3,4] Thus, there is a strong need to investigate alternative therapeutic combinations.

PDT is an alternative CC treatment modality that has been proven to treat primary CC, as well as eradicate CCSCs to prevent secondary metastasis.[5] Since PDT is a very specific non-invasive localized treatment, with very few side effects and it has a short healing time with no scarring, as well as being suitable for repeated dosing, with little to no resistance being found, it seems to be a far more advantageous treatment for CC.[6] Current clinical phase studies by Gunaydin et al. (2021), Li et al. (2020), Afanasiev et al. (2021) and van Straten et al. (2017) have reported that CC ALA PS PDT has emerged as the most effective and harmless treatment strategy for the current control of CC, however it requires further investigation before this treatment can be marketed.[7-10] Additionally, even though the latest CC PDT clinical trials have shown the enormous potential, other preclinical trials indicate that there is still room for improvement.[30] These CC PDT clinical trials improvements include investigating controlled high dosing, refining light source delivery to induce deep tissue phototoxicity with limited skin photosensitivity, as well as enhanced abilities to activate specific immune responses to be able to fully eradicate secondary spread.[11]

This realization has pushed CC PDT and conventional treatment research into the forefront of investigating combination synergistic therapies, which allow for more than one cell death pathway to targeted, in order to strive for both the elimination of primary tumor growth, and the activation of host immune system responses to aid in combating secondary metastasis.[41] Recently, studies by Nkune et al (2021 and 2022) and Mokoena et al. (2019) have suggested the combinative PDT primary treatment of colorectal and breast cancer tumors with CBD in order to ensure complete primary tumor ablation, as well as activate specific immune responses to limit migratory secondary spread.[12-14]

According to *in vitro* CC treatment studies performed by Ramer (2010 and 2008), Contassot et al. (2004), Eichele et al. (2009) and Lukhele and Motadi (2016), it was noted that CBD is able to activate specific immune responses, which can aid in the treatment the primary, as well as secondary treatment of CC.[15-19] This CC metastasis decrease was attributed to CBD's ability to upregulate TIMP-1 and p38 via CB1/CB2 receptor activation and downregulate TRPV1 signaling pathways with Ca^{2+} influx, as well as increase the accumulation of GRP receptors with an upregulation of p53, caspase 3 and Bax, and downregulation of Bcl-2 expression and so induce apoptotic tumor cell death along with angiogenesis, hindering CC primary growth and migratory invasion.[15-19] Thus, CBD may be an effective therapeutic for the primary as well as secondary treatment of CC since it is able to induce apoptotic forms of cell death, as well as decrease tumor cell proliferation, invasion, angiogenesis, and overall metastatic spread. However, studies by Luschnig and Schicho (2019)[39] have noted that further *in vivo* and clinical trial studies need to be performed in order to justify the use of CBD as treatment option for CC. Moreover, studies by Velasco et al. (2016)[40] suggest research studies should be more focused on the combination of CBD with other approved anticancer therapies, in order to assist in overcoming resistance mechanisms, since CBD may promote these single-agent strategies, as well as act synergistically to reduce tumor growth and metastatic spread.

Lastly, even though studies by Luschnig and Schicho (2019) found that CBD could be considered an anticarcinogenic treatment for *in vitro* CC,[39] studies by Liu et al. (2020) reported that tetrahydrocannabinol (THC) promoted the progression of HPV-positive head and neck squamous cell carcinomas via p38 MAPK immune modulation activation and so enhanced cancer growth and metastasis by suppressing antitumor immune response in *in vitro* and *in vivo* models.[53] Since HPV persistent infections in women over the age of 35 years old is responsible for approximately 70% of all diagnosed CC worldwide,[20] the possible treatment of CC with CBD needs to be carefully researched. Even though studies by D'Souza et al. (2010)[54] have stated that cannabis use is not associated with cervical HPV natural history or cervical neoplasia in HIV-seropositive or HIV-seronegative women and so the utilization of CBD or *Cannabis sativa* crude extracts (which contain THC), would have a negligible effect on CC contracted by HPV, researchers need to carefully ensure that this form of CBD treatment could not possibly promote other forms of cancers in patients (such as head and neck cancers), while being utilized to kill cervical cancer cells.

Thus, to promote the effectiveness of both conventional and alternative PDT CC treatment strategies, as well as being able to overcome each of their own limitations and disadvantages, especially in relation to CC resistance

mechanisms and aggressive metastatic spread, more *in vivo* and clinical trial research studies need to be conducted that investigate the synergistic combination of CBD with PDT treatment forms to effectively eradicate CC. However, further studies should also take into consideration and examine how HPV expression is altered by *Cannabis sativa* or its individual components (CBD and THC) in different tissues to produce stronger data to determine if CBD or *Cannabis sativa* crude extracts (which contain THC) are helpful or harmful in terms of aggravating other cancer types.[53]

Author contributions

All authors contributed equally in terms of the studies design and conceptualization. Dr Radmila Razlog and Dr Cherie Ann Kruger were responsible for acquisition, analysis, and interpretation of work, as well as for drafting and revising the work for critically important intellectual content and Prof Heidi Abrahamse was responsible for final editing and approval of this version to be published.

Availability of data

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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ORCID iD

Cherie Ann Kruger https://orcid.org/0000-0002-4556-9132

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71:209-249.
2. Yang L, Shi P, Zhao G, et al. Targeting cancer stem cell pathways for cancer therapy. *Signal Transduct Target Ther*. 2020;5:8.
3. Chao X, Song X, Wu H, You Y, Wu M, Li L. Selection of treatment regimens for recurrent cervical cancer. *Front Oncol*. 2021;11:618485.
4. Mousavi H SR, Bannazadeh Baghi N H, Signaling pathways in cervical cancer chemoresistance: are microRNAs and long-noncoding RNAs the main culprits? Preprints. 2020;2020040294. doi: 10.20944/preprints202004.0294.v1.
5. Chizenga EP, Chandran R, Abrahamse H. Photodynamic therapy of cervical cancer by eradication of cervical cancer cells and cervical cancer stem cells. *Oncotarget*. 2019;10:4380-4396.
6. Ivanova VA, Verenikina EV, Nikitina VP, et al. Photodynamic therapy for preinvasive cervical cancer. *J Clin Oncol*. 2020;38:6035-6035.
7. Gunaydin G, Gedik ME, Ayan S. Photodynamic therapy for the treatment and diagnosis of *Cancer*-A review of the current clinical status. *Front Chem*. 2021;9:686303.
8. Li D, Zhang F, Shi L, Lin L, Cai Q, Xu Y. Treatment of HPV infection-associated low grade cervical intraepithelial neoplasia with 5-aminolevulinic acid-mediated photodynamic therapy. *Photodiagnosis Photodyn Ther*. 2020;32:101974.
9. Afanasiev M, Dushkin A, Grishacheva T, et al. The multi-course approach of photodynamic therapy to treat invasive cervical cancer IB2: a case report. *Case Rep Oncol*. 2021;14:506-519.
10. van Straten D, Mashayekhi V, de Bruijn HS, Oliveira S, Robinson DJ. Oncologic photodynamic therapy: basic principles, current clinical status and future directions. *Cancers*. 2017;9:19.
11. Agostinis P, Berg K, Cengel KA, et al. Photodynamic therapy of cancer: an update. *CA Cancer J Clin*. 2011;61:250-281.
12. Nkune NW, Kruger CA, Abrahamse H. Possible enhancement of photodynamic therapy (PDT) colorectal cancer treatment when combined with cannabidiol. *Anticancer Agents Med Chem*. 2021;21:137-148.
13. Nkune NW, Kruger CA, Abrahamse H. Synthesis of a novel nanobioconjugate for targeted photodynamic therapy of colon cancer enhanced with cannabidiol. *Oncotarget*. 2022;13:156-172.
14. R Mokoena D, P George B, Abrahamse H. Enhancing breast cancer treatment using a combination of cannabidiol and gold nanoparticles for photodynamic therapy. *Int J Mol Sci*. 2019;20:4771.
15. Ramer R, Merkord J, Rohde H, Hinz B. Cannabidiol inhibits cancer cell invasion via upregulation of tissue inhibitor of matrix metalloproteinases-1. *Biochem Pharmacol*. 2010;79:955-966.
16. Ramer R, Hinz B. Inhibition of cancer cell invasion by cannabinoids via increased expression of tissue inhibitor of matrix metalloproteinases-1. *J Natl Cancer Inst*. 2008;100:59-69.
17. Contassot E, Tenan M, Schnüriger V, Pelte MF, Dietrich PY. Arachidonyl ethanolamide induces apoptosis of uterine cervix cancer cells via aberrantly expressed vanilloid receptor-1. *Gynecol Oncol*. 2004;93:182-188.
18. Eichele K, Ramer R, Hinz B. R(+)-methanandamide-induced apoptosis of human cervical carcinoma cells involves a cyclooxygenase-2-dependent pathway. *Pharm Res*. 2009;26:346-355.
19. Lukhele ST, Motadi LR. Cannabidiol rather than Cannabis sativa extracts inhibit cell growth and induce apoptosis in cervical cancer cells. *BMC Complement Altern Med*. 2016;16:335.
20. Hull R, Mbele M, Makhafola T, et al. Cervical cancer in low and middle-income countries. *Oncol Lett*. 2020;20:2058-2074.

21. Tewari KS, Monk BJ. Evidence-based treatment paradigms for management of invasive cervical carcinoma. *J Clin Oncol*. 2019;37:2472-2489.
22. Finocchario-Kessler S, Wexler C, Maloba M, Mabachi N, Ndikum-Moffor F, Bukusi E. Cervical cancer prevention and treatment research in Africa: a systematic review from a public health perspective. *BMC Womens Health*. 2016;16:29.
23. PDQ Adult Treatment Editorial Board. PDQ Cancer Information Summaries [Internet]. Bethesda (MD): National Cancer Institute (US); 2002. Accessed October 6, 2021. https://www.ncbi.nlm.nih.gov/books/NBK66058/.
24. Mumba JM, Kasonka L, Owiti OB, et al. Cervical cancer diagnosis and treatment delays in the developing world: Evidence from a hospital-based study in Zambia. *Gynecol Oncol Rep*. 2021;37:100784.
25. Chen J. The cell-cycle arrest and apoptotic functions of p53 in tumor initiation and progression. *Cold Spring Harb Perspect Med*. 2016;6:a026104.
26. Kwiatkowski S, Knap B, Przystupski D, et al. Photodynamic therapy - mechanisms, photosensitizers and combinations. *Biomed Pharmacother*. 2018;106:1098-1107.
27. Zhang W, Zhang A, Sun W, Yue Y, Li H. Efficacy and safety of photodynamic therapy for cervical intraepithelial neoplasia and human papilloma virus infection: a systematic review and meta-analysis of randomized clinical trials. *Medicine*. 2018;97:e10864.
28. Park YK, Park CH. Clinical efficacy of photodynamic therapy. *Obstet Gynecol Sci*. 2016;59:479-488.
29. Inada NM, Buzzá HH, Leite MFM, et al. Long term effectiveness of photodynamic therapy for CIN treatment. *Pharmaceuticals*. 2019;12:107.
30. Algorri JF, Ochoa M, Roldán-Varona P, Rodríguez-Cobo L, López-Higuera JM. Photodynamic therapy: a compendium of latest reviews. *Cancers*. 2021;13:4447.
31. Stasiłowicz A, Tomala A, Podolak I, Cielecka-Piontek J., Cannabis sativa L. as a natural drug meeting the criteria of a multitarget approach to treatment. *Int J Mol Sci*. 2021; 22:778.
32. Ferber SG, Namdar D, Hen-Shoval D, et al. The "Entourage Effect": terpenes coupled with cannabinoids for the treatment of mood disorders and anxiety disorders. *Curr Neuropharmacol*. 2020;18:87-96.
33. Jin D, Dai K, Xie Z, Chen J. Secondary metabolites profiled in cannabis inflorescences, leaves, stem barks, and roots for medicinal purposes. *Sci Rep*. 2020;10:3309.
34. Morales P, Reggio PH, Jagerovic N. An overview on medicinal chemistry of synthetic and natural derivatives of cannabidiol. *Front Pharmacol*. 2017;8:422.
35. Aliferis KA, Bernard-Perron D. Cannabinomics: Application of Metabolomics in *cannabis* (*Cannabis sativa* L.) Research and Development. *Front Plant Sci*. 2020;11:554.
36. Javid FA, Phillips RM, Afshinjavid S, Verde R, Ligresti A. Cannabinoid pharmacology in cancer research: A new hope for cancer patients? *Eur J Pharmacol*. 2016;775:1-14.
37. Seltzer ES, Watters AK, MacKenzie D Jr, Granat LM, Zhang D. Cannabidiol (CBD) as a promising anti-cancer drug. *Cancers*. 2020;12:3203.
38. Zhelyazkova M, Kirilov B, Momekov G. The pharmacological basis for application of cannabidiol in cancer chemotherapy. *Pharmaciana*. 2020;67:239-252.
39. Luschnig P, Schicho R. Cannabinoids in gynecological diseases. *Med Cannabis Cannabinoids*. 2019;2:14-21.
40. Velasco G, Sánchez C, Guzmán M. Anticancer mechanisms of cannabinoids. *Curr Oncol*. 2016;23:S23-S32.
41. Go YY, Kim SR, Kim DY, Chae SW, Song JJ. Cannabidiol enhances cytotoxicity of anti-cancer drugs in human head and neck squamous cell carcinoma. *Sci Rep*. 2020;10:20622.
42. Dariš B, Tancer Verboten M, Knez Ž, Ferk P. Cannabinoids in cancer treatment: therapeutic potential and legislation. *Bosn J Basic Med Sci*. 2019;19:14-23.
43. Lu HC, Mackie K. An introduction to the endogenous cannabinoid system. *Biol Psychiatry*. 2016;79:516-525.
44. Ayakannu T, Taylor AH, Willets JM, Konje JC. The evolving role of the endocannabinoid system in gynaecological cancer. *Hum Reprod Update*. 2015;21:517-535.
45. Taylor AH, Tortolani D, Ayakannu T, Konje JC, Maccarrone M. (endo)cannabinoids and gynaecological cancers. *Cancers*. 2020;13:37.
46. Afrin F, Chi M, Eamens AL, et al. Can hemp help? Low-thc cannabis and non-THC cannabinoids for the treatment of cancer. *Cancers*. 2020;12:1033.
47. Atalay S, Jarocka-Karpowicz I, Skrzydlewska E. Antioxidative and anti-inflammatory properties of cannabidiol. *Antioxidants*. 2019;9:21.
48. Laezza C, Pagano C, Navarra G, et al. The endocannabinoid system: a target for cancer treatment. *Int J Mol Sci*. 2020;21:747.
49. Das S, Kaul K, Mishra S, Charan M, Ganju RK. Cannabinoid signaling in cancer. *Adv Exp Med Biol*. 2019;1162:51-61.
50. Alvarado-Ortiz E, de la Cruz-López KG, Becerril-Rico J, Sarabia-Sánchez MA, Ortiz-Sánchez E, García-Carrancá A. Mutant p53 gain-of-function: role in cancer development, progression, and therapeutic approaches. *Front Cell Dev Biol*. 2020;8:607670.
51. Burstein S, Salmonsen R. Acylamido analogs of endocannabinoids selectively inhibit cancer cell proliferation. *Bioorg Med Chem*. 2008;16:9644-9651.
52. Hinz B, Ramer R. Anti-tumour actions of cannabinoids. *Br J Pharmacol*. 2019;176:1384-1394.
53. Liu C, Sadat SH, Ebisumoto K, et al. Cannabinoids promote progression of HPV-positive head and neck squamous cell carcinoma via p38 MAPK activation. *Clin Cancer Res*. 2020;26:2693-2703.
54. D'Souza G, Palefsky JM, Zhong Y, et al. Marijuana use is not associated with cervical human papillomavirus natural history or cervical neoplasia in HIV-seropositive or HIV-seronegative women: table 1. *Cancer Epidemiol Biomarkers Prev*. 2010;19:869-872.