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The Development of Locoregional Therapies as a Strategy for Reducing Cervical Cancer Mortality in Low to Middle Income Countries.

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Abstract

Early detection concomitant with appropriate treatment intervention for pre-invasive cervical cancer has proved effective in the 'war on cervical cancer' in the developed world. Unfortunately, these advances have not been mirrored in the developing world, where incidence and mortality rates are currently approximately 90% of the world's burden. Due to economic, infrastructure and skilled personnel constraints, the impact of cytology screening as a method for early detection and reducing mortality rates from cervical cancer is lower. Typically, women present with advanced disease that is difficult to treat because of de novo and pharmacokinetic resistance. Whilst the HPV vaccine is a welcome development in the fight against cervical cancer, for women who are outside the target age of vaccination, or indeed do not have access to vaccination, screening remains the only form of protection. Current excisional treatments available for cervical dysplasia are effective but have limitations, including increased incidence of obstetric complications and risk of recurrence. This is a particular issue in cases of HIV, which is endemic in the regions most affected by cervical cancer. Therefore, early detection combined with early treatment is an attractive strategy to reduce the number of women presenting with drug resistant disease in developing countries where cytology screening and vaccination services are poorly developed. This review makes the case for developing a locoregional treatment therapy for cervical dysplasia which could be incorporated into a cervical cancer screening strategy in a rural setting within a developing country.

Introduction

Where a woman lives, plus her socioeconomic status, largely determines whether she will develop cervical cancer, how early she presents to healthcare services, and her access to affordable, good quality diagnostic and treatment services [1]. Cervical cancer is preventable because it has a long pre-invasive phase that is easily identified by clinical and histopathological examination. The incidence of this disease is therefore directly related to a nation's medical infrastructure and the resources available for population-wide screening and the treatment of identified cancers. Prevention and early detection offer the most cost-effective, long-term strategy for the control of cancer, even in low resource settings. Proven and cost-effective interventions for cervical cancer are available, yet access to these are beyond reach for many women in low and middle-income countries (LMIC). These countries have fragmented health systems and are responsible for 86% of the world's cervical cancer cases [2]. Shockingly, only 5% of global spending on cancer is directed towards them [3]. Without significantly increased screening and preventive treatment services, an estimated 19 million women will die from cervical cancer over the next 40 years [4]. A key reason for the high mortality rate is the fact that women typically present with late stage or advanced disease. Under these circumstances, treatment is significantly compromised by multiple issues including*de novo* and pharmacokinetic (poor drug

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delivery and penetration) resistance. In this review, we discuss the potential benefits that could be gained by use of a 'screen and treat' strategy using simple screening methods and locoregional therapies to detect and treat cervical cancers early. The aim would be reducing the number of women in LMIC presenting with advanced, drug resistant disease. It is therefore pertinent to review the magnitude of the problem in LMIC and evaluate the limitations of current treatments for cervical cancer.

The Scale of the Problem in Sub-Saharan Africa

In 2018, there were an estimated 570,000 cases and 311,000 deaths, from cervical cancer worldwide [5]. It is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer death in women worldwide. Cervical cancer is the most commonly diagnosed cancer in 28 countries and the leading cause of cancer death in 42 countries. The majority of these countries are in Sub-Saharan Africa and South-Eastern Asia, with the highest incidence rate in Swaziland, and the highest mortality rates found in Malawi, Zimbabwe, Guinea, Burkina Faso, and Mali [5]. In these countries, incidence and mortality rates are seven to 10 times higher than in North America, Australia/New Zealand, Saudi Arabia and Iraq [5].

The development of a country can be measured using the Human Development Index (HDI), using its people and their capabilities as opposed to economic growth alone. HDI measures three basic dimensions of human development [6] (i) life expectancy at birth (ii) the average number of years of schooling achieved by adults aged 25 years and above and (iii) the gross national income per capita. There appears to be a direct relationship between the HDI of a country and incidence of cervical cancer. When the national incidence burden of cervical cancer is compared the country's HDI level, cervical cancer makes up to a third of all cancers diagnosed (in both sexes) in many low HDI countries. By comparison, cervical cancer comprises less than 10% of all cancers in very high HDI countries [1]. For this reason cervical cancer can be considered "a case study in health equity" [7] based on the unequivocal evidence that cervical cancer is a major problem in LMIC, especially Sub-Saharan countries. Among the most cost-effective strategies according to WHO's Global Action Plan for the Prevention and Control of Non-Communicable Diseases (2013–20) [8]. is screening with treatment of pre-cancerous lesions. However, access to these strategies varies depending on where a woman lives, her socioeconomic status and her agency. In 2020, the WHO assembly adopted the Global Strategy for Cervical Cancer Elimination, the aim of which is to eliminate cervical cancer by 2030 through three main approaches: vaccination with a target of 90% of girls fully vaccinated against HPV by the age of 15; screening with a target of 70% of women screened by the ages of 35 and 45; treatment with the target of 90% of women with pre-cancer treated and 90% of women with invasive cancer managed [9]. This can only be achieved if comprehensive cancer control is implemented in LMICs, including universal access to early diagnosis and accessible treatment for cancer. Therefore, the need for effective strategies to reduce the incidence of cervical cancer, whilst boosting early detection, and the application of treatments (existing and novel) for this disease are imperative in LMIC.

VIA as a Screening Test for Early Detection of Cervical Cancer

The visual inspection with acetic acid (VIA) screening method developed in 2000 has proved to be a valuable tool for cervical cancer screening that developing countries so desperately need. The attractiveness of this simple screening method is based on its ability to be incorporated into a linked 'screen and treat' strategy. Integrating screening and treatment that bypasses the need for a protracted wait for screening results and potential loss of follow-up appointments, which is endemic in LMIC countries. Further advantages are the low cost, ability to be performed by trained nurses and lack of need for laboratory facilities or diagnostic equipment. While VIA is more sensitive than cytology with a sensitivity of 76·7% compared to 44·3% for cytology, it is less specific with a specificity of 64·1% compared to 90·6% for cytology[10]. Despite this, VIA remains an attractive option, especially in rural areas, where medical and pathology services are not well developed. Unfortunately, to date VIA has been slow in being rolled out in developing countries (especially in the rural areas where it is needed most) as the average screening coverage in Sub-Saharan Africa is only 12.12% [11]

Current Treatments for Cervical Dysplasia and their Limitations

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Treatments for pre-cancerous cervical lesions developed over the past five decades, are responsible for reducing the incidence and mortality of cervical cancer in developed countries over the same period of time[12,13] (Kitchener et al., 2006; Peto et al., 2004). Essentially, treatment uses ablative methods such as cryotherapy, laser ablation and electrocautery. Alternatively, excisional methods such as loop electrosurgical excision procedure (LEEP) (or large loop excision of the transformation zone (LLETZ)) and cold knife cone biopsy (CKC) (also known as conization) can be used, depending on the extent and severity of the lesion. Ablative methods destroy the precancerous cells in the transformation zone by necrosis, with no anaesthetic required, and no tissue is removed. Excisional methods involve removal of the precancerous lesion under either local or general anaesthetic, together with a margin of healthy tissue, which can be sent for histological evaluation. LEEP and CKC are performed by an experienced clinician, generally an obstetrician/gynaecologist who can recognize and manage complications, and in a facility where surgical back-up is available if required [14]. This limits the use of excisional methods as treatments in LMICs [14]. Whilst these surgical procedures remove the neoplasia, they do not target the cause of these lesions, which is persistent HPV infection. Recurrence rates for cervical intraepithelial neoplasia (CIN) after treatment is between 5-26% [15,16].

There are advantages and disadvantages associated with each of the techniques used, for example there is an increase in major and minor bleeding with LEEP compared to CKC, however there is a decreased risk of major infection with LEEP compared with CKC [17]. CKC has the lowest recurrence rate of CIN 2+ when compared with LEEP and cryotherapy [17]. However, CKC is associated with other complications such as major infection (including pelvic inflammatory disease) and increased risk of premature labour [17]. A meta-analysis on perinatal mortality and adverse pregnancy outcomes showed that cold knife conization, laser conization and radical diathermy were associated with a significantly increased risk of peri-natal mortality, pre-term delivery, low birthweight[18,19], premature rupture of membranes and caesarean section [18]. While the literature is not consistent about effects of excisional therapy on fertility, and a meta-analysis[20] found no evidence to support an effect on fertility outcomes, there was evidence that excisional therapy increased the risk of a second trimester miscarriage possibly due to cervical incompetence after large excisions.

A further complication in LMIC is that while excisional therapy is effective treatment for cervical dysplasia in immunocompetent patients, it seems to be effective only in slowing progression to cervical cancer in HIV positive women. The recurrence rates for women with HIV are much higher than for HIV negative women, even in the Highly Active Anti-retroviral Therapy (HAART) era, with studies showing recurrence rates of up to 63% [21–24]. Fruchter et al., reported that 90% of HIV-infected women with CD4 counts less than 200 cellsmm^{-3} developed recurrent dysplasia at 3 years[21]. While antiretroviral therapy may delay the recurrence of HPV-related disease in HIV positive women, immune restoration on HAART is insufficient to clear HPV because HPV DNA persists in patients on HAART [25]. Furthermore, the incidence of invasive cervical cancer has remained constant since the introduction of HAART [26], and treatment with HAART has failed to eradicate CIN in many HIV-infected women [27]. As a result, definitive management with hysterectomy has been advocated for eradication of cervical dysplasia in HIV positive women.

Recent data suggests that most CIN2 lesions regress spontaneously, particularly in young women, under 30 years [28]. This means that the reclassification of CIN2 and CIN3 as high grade squamous intraepithelial lesion (HSIL) has huge implications for many women, especially young women in their reproductive years. There is a possibility that spontaneously regressing lesions could be classified as high grade lesions that warrant treatment and the attendant increased risks of reproductive morbidity [1817]. Taken together, there is a clear case for developing locoregional medical therapies in LMIC based on the "screen and treat" approach. Simple screening using VIA combined with application of the treatment directly to the surface of the cervix, would circumvent the issues of delayed diagnosis, low follow-up rates, the high risk of infection or adverse pregnancy outcomes attendant with current practice.

Chemoresistance in Cervical Cancer Therapy

Currently, invasive cervical cancer is treated with surgery or radiation combined with a platinum-based chemotherapy. The most effective chemotherapeutic agent used in cervical cancer treatment is Cisplatin, which can be used either as a single agent or in combination with other therapeutic agents, e.g. 5-Fluorouracil

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[29]. More advanced stages of cervical cancer are treated by chemotherapy, including metastatic disease, as well as patients with persistent, or recurrent disease. Unfortunately these patients typically have a poor prognosis because cancer cells can develop resistance to the chemotherapy agents [30]. One of the main obstacles in the treatment of late-stage cervical cancer is resistance which develops to chemotherapeutic agents [31]. An increase in the number of available chemotherapeutic agents has resulted in increased resistance in cancer cells as more exposure to medications makes tumour cells more prone to developing drug resistance which subsequently leads to widespread metastasis [29].

Cisplatin interacts with DNA molecules to form cross-linked DNA "adducts" which in turn activate several downstream molecular pathways including p53 and MAPK, ultimately resulting in apoptosis[29]. Chemoresistance arises in cancer cells via the downregulation of tumour suppressors and the stabilization or activation of cell survival factors [32] including increased DNA repair, decreased uptake of drug into the tumour cells and increased inactivation of drug [29]. These mechanisms lead to less apoptosis in cancer cells, resulting in unchecked tumour replication and spread of malignancy [33,34].

Most women in Africa present with late-stage cervical cancer when chemo-radiotherapy becomes the only option for curative or palliative treatment. A recent study of 2760 patients from 11 African countries found that 65.8% had stage III-IV disease [35]. In Zimbabwe, 76% of women present at the later stages of the disease[37]. Patients who present with late stage disease have poorer outcomes. The 5-year overall survival for stage III-IV cervical cancer as determined from nine African registries was 20.5%[36] compared to 50.3% for stage I-II cervical cancer[35]. Moreover, it is estimated that only 8% of The International Federation of Gynaecologists and Obstetricians (FIGO) stage I-III patients in Africa receive guideline-adherent care due to lack of access to treatment facilities. While an estimated 55% of new of cancer diagnoses in Africa could be treated by radiotherapy, there are no available radiation facilities in 26 of 54 African countries[38]. In countries where they do exist, they are inaccessible to most patients because they are either located in tertiary institutions, are non-functional or poorly maintained, or they are available only in the private sector at a cost out of reach for most patients [38]. Late-stage disease presentation in addition to poor access to radiotherapy means that therapeutic options for advanced presentations are increasingly limited to chemotherapy alone, further compounding the problem of chemoresistance.

Overcoming drug resistance is an important approach in cancer research, and various agents including phytochemicals have been investigated as potential bioactive agents in the arsenal of the war against cancer. For example, curcumin sensitizes cervical cancer cells to paclitaxel treatment and inhibits pathways involved in cellular proliferation and survival *in vivo* [39]. It also plays a role in the reversal of resistance to cisplatin in cervical cancer cells[40]. An indirect but logical way of mitigating the effects of multi drug resistance in cervical cancer is to focus on early detection of cervical lesions and early intervention to prevent progression to late-stage disease. This strategy has a three-fold advantage. Firstly, targeting early lesions which have not breached the basement membrane and are contained to the epithelium offer a pharmacokinetic advantage for a loco-regional therapy as drug will be penetrating a thin layer of cells with less chance of pharmacokinetic drug resistance developing. Secondly, treating the disease at the early stages negates the use of chemotherapy and hence abrogating the factors that lead to the development of chemoresistance. Thirdly, and more pertinent to LMIC treating early-stage disease removes the financial and resource burden associated with treating late-stage disease, thus making the disease eminently more treatable with rapid and better patient outcomes.

The concept for developing a locoregional therapy for cervical cancer in LMIC would focus treatment directly onto the pre-neoplastic lesion leading to local control of the disease and effectively preventing recurrence. It would be particularly applicable in the case of HIV positive women for whom treatment is associated with high recurrence rates, and for whom treatment resistance is a problem. In a country like Zimbabwe where HIV prevalence rates are around 15%[41], this would apply to a significant proportion of women. Locoregional therapy as part of a "screen and treat" strategy would also reduce the need for yearly cervical screens following a diagnosis and treatment of high-grade cervical dysplasia (CIN2+). Importantly, any treatment that negates the use of excisional or ablative methods would be beneficial as it would also reduce the incidence of obstetric

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complications, in women of reproductive age. There is a clear rationale for developing loco-regional therapies for treating cervical cancer, particularly in the rural areas of LMIC where access or travel to medical facilities can be problematical.

Precedence for Locoregional Therapies to treat Cervical Dysplasia

It is well established that cervical cancer is caused by persistent infection with one or more of the oncogenic human papilloma virus (HPV) genotypes for a period longer than 2 years [42,43]. HPV infection is common worldwide, with a lifetime cumulative risk of greater than 80% [44]. To date only a few drug-based locoregional therapies have been developed for HPV infection which are approved for use in clinical practice, however, none of them are licensed for the management of cervical dysplasia. These therapies target either the molecular virology of HPV infection, the neoplasia itself, or the immune response to the virus (or indeed all three modalities) leading to viral clearance or lesion elimination[45]. Strategies that target the HPV virus aim to reduce the rate of recurrence by focusing on the cause of the lesions and thereby have an impact on the amount of latent and subclinical HPV DNA rather than just removing the neoplasia [45]. There is precedence in the literature for locoregional therapies to treat cervical dysplasia either indirectly by targeting the HPV infection or directly by treating the cancer. Details of treatments so far trialed have been reviewed extensively elsewhere [46]. Briefly, topical therapies studied so far have included immune modulators such as imiquimod [47–49], antiproliferative therapies such as 5Fluorouracil (5-FU) [50,51], antivirals such as Cidofovir [52], Lopinavir and Ritonavir, a combination oral HIV protease inhibitor (Lopimune) [53–55], Vidarabine [56,57] and Terameprocol[57,58] and hormonal drugs for treatment of CIN 1 such as Dehydroepiandrosterone (DHEA) [59]. In addition, other approaches include herbal/non-pharmacologic therapies such as green tea [60], praneem, the seed extract of*Azadirachta indica* [61], glycyrrhizinic acid[62] and curcuma (turmeric). Of all these, the randomized trials of Imiquimod, 5-FU, and Cidofovir have had the most promising results [46]. These will be discussed in more detail in the ensuing review.

Designing a Cervico-vaginal drug delivery system

The cervical and vaginal ecosystem is complex due to the balance between the conflicting need for protection against infection and allowing entry of sperm to the upper genital tract. As such, there is a pH gradient from the acidic vaginal canal at pH 3.8 - 4.5 [63–65]to the alkaline cervical mucus at pH 7.0 [66]. The accessibility of the cervix and presence of a good blood supply makes vaginal drug delivery an ideal potential target for a locoregional therapy. In addition, this route avoids both hepatic first pass metabolism as the absorbed drugs enter directly into the systemic circulation and gastrointestinal enzymatic degradation associated with the oral route [67]. Vaginal drug delivery systems in current use comprise liquid forms including foams, douches and irrigations, semi-solid forms such as creams, gels and ointments, and solid forms in the form of pessaries, rings and films[68]. All these have limited efficacy depending on their residence time at the genitourinary tract [69]. A vaginal drug delivery system designed for locoregional therapy should ideally, distribute uniformly and retain the drug at the administered the site of action for a prolonged period [69]. This depends upon the properties of the delivery system, and vaginal physiology. Factors relating to vaginal physiology include the volume, viscosity and pH of vaginal fluid and the thickness and porosity of the epithelial layer which in turn varies with age and menstrual cycle[70]. The self-cleansing action of the vaginal tract[71,72] results in a decrease in the therapeutic effects of these delivery systems due to reduced drug bioavailability[73]. Furthermore, the protective mechanism of the genitourinary tract further limits residence time, making it necessary for multiple and frequent drug applications for effective treatment[69]. This would be further impacted by the additional challenge of patient compliance if repeated applications are required for treatment.

Pessaries in the form of tablets or suppositories provide a sustained release of the drug as they gradually dissolve [68]. The bioavailability of the drug depends upon its residence time in the vaginal canal [69]. Creams are typically emulsions whereas gels are three-dimensional hydrophilic polymers made up of long, disordered chains with reversible cross-links [74]. A potential issue with using both creams and gels is that they do not provide an exact dose and they can be messy, uncomfortable and cause leakage [75]. Patients generally tolerate gels better than other dosage forms [76].

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Over the last decade, muco-adhesive and *in situ* gelling formulations have been used to increase the residence time of liquid vaginal drug formulations at the site of action [77]. Muco-adhesive polymers such as polyacrylic acid-based Carbomers interact with mucus proteins in the vaginal cavity by forming weak hydrogen bonds or disulphide bonds with thiol groups, thereby prolonging the formulation residence time at the action site [73].*In-situ* gelling systems are typically present in a sol-state at room temperature, which describes a type of colloid with small particulate solids dispersed within a liquid, before administration. They subsequently undergo a sol–gel transition in response to temperature, pH change or the presence of ions upon contact with biological fluids or mucous membranes in the vaginal canal, forming a gel. The gel releases the loaded drug in a controlled manner, resulting in a formulation with reduced administration frequency, ease of administration and therefore improved patient compliance and comfort[77]. They also include solid formulations such as polymeric matrices and films that undergo fast hydration in biological fluids to form a gel with controlled release properties. Nanosystems can also be loaded onto a muco-adhesive *in-situ* gelling vehicle. The intimate interface between the mucus and gel enables transfer of the nanosystems to the site of action on the vaginal mucosa[78].

Locoregional therapy for cervical lesions

As mentioned previously, none of the drug-based locoregional therapies so far developed for HPV infection are licensed for the management of cervical dysplasia. There have, however, been some promising results from trials of locoregional therapy for cervical lesions. We will now discuss in greater detail some of these studies involving 5-Flourouracil, Cidofovir and more recently, Lopimune, including some of the advantages, limitations, and the potential for clinical use of the various therapies.

5-Flourouracil

5-fluorouracil (5-FU) is an antimetabolite analogue of the pyrimidine uracil, with a fluorine atom at the C-5 position in place of hydrogen[79]. Intracellularly, the fluoropyrimidine is converted into fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP) which become mis-incorporated into RNA and DNA [80]. Furthermore, FdUMP inhibits the enzyme thymidylate synthase by competitively binding to its active site thereby blocking binding of the normal substrate dUMP and inhibiting dTMP synthesis [81,82]. Because of its cytotoxicity, 5-FU is used widely as a chemotherapeutic agent in cancer treatment. Topical 5-FU cream is used for treatment of intraepithelial neoplasia of the skin and off-license treatment of HPV related lesions including genital warts, vulvar intraepithelial neoplasia and vaginal intraepithelial neoplasia [83,84].

The safety profile of 5-FU was established in a randomized controlled study involving 90 women with HPV-associated vaginal and vulval lesions. Women who used prophylactic topical 5–FU at a dosage of 2 g once every two weeks after ablative therapy, were significantly less likely to develop recurrent lesions than controls [85]. In a later study where the drug was used for vaginal HPV-associated lesions continuously over five to seven days, or periodically once a week for 10 weeks, dose and duration dependent chronic mucosal ulceration developed in 8.2% of subjects [86]. In a separate study, 5-FU was used as an adjunctive treatment following excisional therapy for CIN 2-3 in HIV positive patients in order to prevent recurrence[50]. A dosage of 2 g of 5% 5-FU cream was self-administered using vaginal applicators at bedtime once every two weeks for six months. The recurrence rate in the treatment arm was 28% compared to 47% in the observation arm and there was a statistically significant prolonged time to recurrence in the treatment arm compared to the observation group. Once again vaginal 5-FU at this dosage and interval was extremely well tolerated, with minimal morbidity in HIV-infected women with CIN [50].

Another trial investigated intravaginal 5% 5-FU vs standard-of-care observation in young women aged 18 – 29 years with CIN 2 with the primary outcome of disease regression at six months after the diagnosis of CIN 2 [51]. Dual contraception with condoms plus one either oral, intravaginal, injectable, implantable, or intrauterine device was required for the 5-FU group because of its potential teratogenic effects. The established dosage of 2 g of topical 5% 5-FU cream was applied once every two weeks for a period of 16 weeks (a total of eight doses) which is standard with other studies[87–90]. This was inserted into the vagina

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at night using a vaginal applicator, and a tampon placed per vagina overnight to keep the cream in-situ, and then removed in the morning. Disease regression was demonstrated in 84% of women in the 5-FU group compared to 52% of women in the observation group, and after six months and the treated group was twice as likely to be both HPV negative and clear of neoplasia [51].

As described above, one of the drawbacks of using a vaginal cream based locoregional therapy is the inability to control the precise site of application and the duration of drug action, resulting in local and systematic side effects [91,92]. In an effort to circumvent this problem, 5-FU was incorporated into a one-way release bilaminar bio-adhesive drug delivery system [93] which was placed directly on the cervix using a device applicator, and kept*in situ* for 24 hours, after which time it was removed using threads that were attached to it for that purpose, releasing a once only dose of 20 mg. This study involved 104 patients with CIN 1 and 2, where the control arm received the delivery system loaded with a placebo. The device was spontaneously expelled in four patients before the 24 hours was up, and 41% of patients in the drug arm developed erythema of the cervix compared to only 8% in the placebo group. More importantly, there was no difference in remission of CIN between drug and placebo groups [93]. The authors concluded that despite the effectiveness of the delivery system, one application of 5-FU was not effective in the treatment of CIN due to inadequate dosage.

In a separate bid to achieve better therapeutic efficacy and increased patient compliance, 5-FU was formulated in a vaginal gel with thermosensitive and muco-adhesive properties in order to increase residence time at the cervix [94]. The drug was incorporated as its inclusion complex in a 1:1 molar ratio with either b-cyclodextrin or hydroxypropyl-b-cyclodextrin in order to increase its aqueous solubility and to achieve the complete release of 5-FU from the gel and this did not affect the gelling temperature significantly[94]. The muco-adhesive polymer HPMC conferred favorable thermosensitive *in situ* gelling properties and complexation to cyclodextrins resulted in a ten-fold increase in cytotoxicity against HeLa cells [94]. This was also confirmed by the enhanced release profile of 5-FU through complexation [94]. All this suggesting that formulating an anticancer drug in a muco-adhesive, thermosensitive gel in complexation with cyclodextrins can increase its anticancer activity resulting in lower doses and reduced unwanted side effects through controlled release and prolonged residence time at the administration site. The disadvantages of 5-FU are its potential for teratogenic effects in its intravenous form in women of child-bearing age and for causing cervical mucosal ulceration [86]thereby potentially placing women at increased risk of sexually transmitted infections [51].

Cidofovir

Cidofovir is an acyclic nucleoside phosphonate with a broad-spectrum activity against DNA viruses. It competitively inhibits the incorporation of deoxycytidine triphosphate into viral DNA by viral DNA polymerase, causing a disruption in chain elongation[95]. Cidofovir's mechanism of action and its selectivity for HPV-transformed cells is dependent on their inability to respond to DNA damage, rather than having a direct anti-HPV effect. Viral oncoproteins E6 and E7, in HPV infected cells cause deregulation of cell cycle control, making these cells are more susceptible to DNA damage than normal keratinocytes [96]. Treatment with Cidofovir causes cell death by apoptosis. Induction of apoptosis in HPV infected cells by Cidofovir was associated with accumulation of the tumour suppressor proteins p53 and pRb and the cyclin-dependent kinase inhibitor p21/WAF-1 [97].

Systemic exposure of Cidofovir after vaginal application is low. This was observed in a trial of nine women with CIN2+ treated with 3 g of 2% Cidofovir gel administered in a cervical cap and applied directly to the cervix, once per week for five or ten hours, over a period of three weeks. Very low plasma concentrations were reached when compared with intravenous administration, at the approved dose [98]indicating that the drug stays at the desired site of action on the cervix and works locally with limited systemic absorption and thereby minimizing the risk of unwanted systemic side effects. In another study, 48 women with biopsy-proven CIN2+ were treated with either three applications of 3 ml of 2% Cidofovir gel in a cervical cap or a placebo for four hours, six weeks before conization. Biopsy results from the excised cones showed resolution of CIN in 60.8% in the Cidofovir group compared to 20% in the placebo group. In addition, there were no systemic toxicity or cervico-vaginal side effects of Cidofovir compared to the placebo. However, Cidofovir failed to eradicate HPV infection[52]. Contrastingly, in a separate study by Snoeck et al., 15 women had 3 g

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of 1% Cidofovir gel applied on the cervix three times on alternate days by a gynaecologist using colposcopy for an average of 17 days. The results from the cone biopsies taken after more prolonged treatment with Cidofovir showed a complete response in seven of the 15 patients and in four of these seven patients, PCR results showed resolution of HPV infection. Once again there was no toxicity observed in the normal tissue and the treatment was well tolerated[99]. These studies support selectivity of Cidofovir against HPV induced epithelial proliferation compared to normal epithelium. In five of the 15 patients there was a limited response in the superficial epithelial layers, two patients did not respond, and one patient downgraded from CIN3 to CIN1. The partial response is likely due to poor bioavailability of the drug at the deeper epithelial layers indicating the need to modify the dosage or formulation of the drug in order to reach the deeper layers [99]

Lopimune

Lopimune is an oral HIV Protease Inhibitor (PI) comprising of Lopinavir and Ritonavir. Pre-clinical and early trial results in the locoregional therapy for CIN have been promising [53]. PIs have anti-cancer activity against HPV- related cervical disease, in addition to their anti-HIV action [54] and Lopinavir is the most effective. It exerts its activity by blocking the HPV16 E6 protein from producing proteasomal degradation of p53 in cultured cervical carcinoma cells [100] and upregulation of the antiviral protein, ribonuclease L, and this has been subsequently confirmed in HPV16 E6/E7 immortalized keratinocytes [54] and at doses used for oral HIV therapy, which are more than ten times the concentration achieved in cervico-vaginal fluid [101], Lopinavir shows high selective toxicity for E6/E7 expressing cells[102]. Lopimune is formulated as an oral gelatine capsule for HIV treatment.

In a single-arm non-randomized proof-of-concept clinical trial done in Kenya, 23 high risk HPV positive women with CIN2+ used Lopimune capsules as a self-applied twice daily vaginal pessary for a period of two weeks[53]. Cytology was done at 12 weeks which was confirmed by histology, and showed that in 77.8% there was either complete resolution of dysplasia or there had been a change from CIN2+ to CIN1/2, and HPV was no longer detected in 52.2% of women. Treatment with Lopimune was well tolerated, and nausea was the only reported adverse reaction. Systemic exposure of the drug was limited, reaching plasma levels of 10ng/ml which is much less than the levels of 2.8-6.6 μgml^{-1} that is reported with oral delivery in HIV therapy [101]. However, this could be partly due to the sub-optimal dosage used in the trial, which resulted in some women showing a transient change in HPV status or cytology which reverted back to baseline after 12 weeks. The authors surmised that since HPV is DNA-based and therefore more stable than RNA-based HIV, it is possible to repeat treatment multiple times with less chance of developing resistance, as a way of addressing treatment failure. In contrast to 5 FU, Lopimune is a non-cytotoxic drug which can be safely used in women of child-bearing age. Additionally, it is currently licensed for systemic treatment of HIV infected pregnant women and children[103].

Potential New Locoregional Treatments for Pre-Invasive Cervical Cancer

The anticancer drug EO9 (or Apaziquone) is an indolequinone based bioreductive drug that requires activation by cellular oxidoreductases to generate DNA damaging species. Its mechanism of action, which centres on the enzyme NQO1 reducing EO9 to reactive species, has been extensively reviewed elsewhere alongside its pre-clinical and clinical history [104,105]. Briefly, EO9 failed clinical trials when administered intravenously primarily because rapid pharmacokinetic elimination prevented sufficient drug reaching the tumour to induce an anti-tumour effect. It was argued that whilst its poor pharmacokinetic properties were not suitable for systemic drug administration, they would paradoxically be ideal for the locoregional treatment of pre-invasive cancers [106]. In the case of non-muscle invasive bladder cancer (NMIBC), intravesical administration would circumvent the drug delivery problem and any drug reaching the systemic circulation would be rapidly cleared thereby reducing the risk of systemic toxicity. In a phase I/II pilot study involving 12 patients with low risk NMIBC, eight complete responses were recorded[107] with similar complete response rates (67%) reported in phase II studies [108]. Furthermore, follow up studies reported good two-year recurrence free rates[109,110] suggesting that the long-term response rates were good in comparison to standard of care treatments for NMIBC. Whilst the results of phase III clinical trials were disappointing (due largely to changes in clinical trial design from phase II to phase III[105]), these studies have nevertheless established

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the principle that compounds with poor systemic pharmacokinetic properties can have clinical efficacy when used in a loco-regional setting. In the context of pre-invasive cervical cancers, NQO1 is overexpressed in squamous cell carcinoma of the cervix and CINs compared to normal cervical epithelia [111] suggesting that EO9 would be a promising candidate for the locoregional treatment of pre-invasive cervical cancer.

Conclusion

Cervical cancer is endemic in sub-Saharan Africa and other parts of the developing world, that are least prepared to deal with a high burden of disease. The current methods of screening and treatment for cervical dysplasia and invasive cancer have clearly not achieved the same results in the developing world as they have done in the developed world towards reducing the burden of this disease. With up to nine out of 10 cervical cancer cases and deaths occurring in LMICs [2] and the disease being the most commonly diagnosed cancer in 28 of these countries and the leading cause of cancer death in 42 countries[5], it has become imperative that innovative ways of dealing with the problem of cervical cancer in sub-Saharan Africa and the developing world are found. Ideally this innovative approach would address the limitations posed by the current methods of treatment for cervical dysplasia, as well as being able to be integrated into the existing VIA screen and treat strategy used in this region, which negates the need for trained clinicians and expensive medical infrastructure. An ideal locoregional therapy for HPV-induced cervical lesions to be used in this setting would be a cytotoxic anticancer agent which would target the pre-neoplastic lesion with high selectivity for diseased epithelium and non-toxic to normal tissue and low systemic absorption. The anticancer agent must be readily available, safe to use in women of child-bearing age and effective. The formulation would be self-administered with increased residence time at the cervix and therefore require a minimum number of applications, for it to be acceptable to patients. In this growing area of research, developing a drug-based locoregional treatment for cervical dysplasia which can be administered at the time of screening by a nurse or even by the patient themselves and with no requirement for specialized equipment, could prove to be the game-changer that sub-Saharan Africa so desperately needs in the fight against cervical cancer.

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Authors' contributions

TC designed, wrote and prepared the article with SJ and RMP contributing to the conceptual design, reviewing and critical appraisal.

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There are no conflicts of interest.

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