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REVIEW ARTICLE

Human papillomavirus: problems and prospects for women's reproductive health (systematic review)

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ABSTRACT

Aim: To consider the specific activity of drops and suppositories of PROTEFLAZID® at the stage of preclinical study, to assess the effectiveness and safety of use in clinical practice in papillomavirus-associated diseases of the female reproductive system.

Materials and Methods: Analysis of scientific publications on the treatment of palilomavirus infection with PROTEFLAZID® in women over the past decade.

Results: Medicines have a direct antiviral effect on papillomaviruses. Clinical trials have shown that the drugs are safe and effective agents for the treatment of papillomavirus-associated CIN1 and CIN2 in the mode of simultaneous systemic and topical use. The proposed scheme is a promising method of treating class I-II neoplasias in women of reproductive age, as it avoids cervical damage, early and late complications and preserves female reproductive function.

Conclusions: PROTEFLAZID® (drops and suppositories) are safe and effective etiopathogenetic drugs for the systemic and topical treatment of papillomavirus-associated diseases in women. Medicines demonstrate the potential for the prevention of cervical cancer, as they allow organ-preserving treatment in women of reproductive age as a guarantee of future motherhood.

KEY WORDS: papillomaviruses, dysplasia, prevention, treatment, PROTEFLAZID®

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INTRODUCTION

Cervix disorders are of particular importance both in the clinical practice of obstetricians and gynaecologists and in the healthcare sector in general. Their incidence is exceptionally high and does not tend to decrease. Early detection and proper treatment of both pre-existing conditions and precancerous cervix disorders can prevent the onset of cervical cancer (CC). Hence, the issue of early diagnosis and treatment of cervix disorders from the perspective of women's reproductive health is crucial, as the absence of symptoms of the condition facilitates its progression.

Multiple investigations by morphologists, oncologists, gynaecologists and immunologists indicate that DNA-containing viruses, namely human papillomaviruses (HPV), are an etiological factor for CC.

The production of intact viral particles characterises the non-integrated form of infection. This is when HPV infection is asymptomatic and extremely pathogenic.

In patients with pre-existing cervix disorders (diseases), HPV infection is common. Therefore, the issue of HPV infection warrants particular attention, given the presence of a viral factor (HPV) that puts such patients at increased risk of CC.

HPV can be classified as a disease that affects the nation's demographics, as the infection is sexually transmitted. In pregnancy, latent HPV becomes active due to changes in the immune system and accelerates the tumour transformation of the cervical epithelium. Infection during pregnancy and childbirth is also possible. Typically, young women and men aged 20-30 years are affected, who consult an oncologist with an advanced disease, since the disease progression to malignancy is slow and almost unnoticeable to patients.

Genital HPV infection is one of the most common sexually transmitted infections [1-33]. Viral infections play a prominent role among reproductive system diseases due to the global deterioration of the population's immune status [34-42]. HPV is a DNA-containing viruse of the family Rapovaviridae and has a tropism for squamous cell carcinoma [35, 38, 39, 41]. Current data indicate that HPV is a conditional pathogen, as evidenced by its diagnosis in 10-30% of women without reproductive system diseases [2, 7, 15, 17]. Spontaneous regression of the virus is possible within 1-2 years (in the case of non-oncogenic types), and in cases of highly oncogenic types, the infection often acquires a persistent character [1]. HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 are considered to be the most highly carcinogenic types [35, 38, 39], which account for 71.5% of cervical cancer cases in Europe [33,38,39,42]. Viral infection was shown to remain latent for several years; however, its presence increases the risk of developing cancer by an average of 300 times; 99.7% of cervical squamous cell carcinoma cases contain human papillomavirus DNA [15, 39, 42]. The pathogenetic foundation of HPV-induced cancer is the integration of viral DNA into the chromosomes of infected cells, active synthesis of viral oncoproteins E_6 and E_7, and disruption of the normal process of apoptosis and cell differentiation [15, 35, 38, 42]. HPV leads to chronic

inflammation of the cervix (HPV-associated cervicitis) in 5-15% of patients [1, 4, 12, 26, 27, 32].

Cervical cancer ranks seventh among the most common types of cancer among women in the European region. Its share in the total cancer incidence is about 3.8% [20], and it is the second most common cancer among women worldwide [9]. Most cases of cervical cancer come from less developed regions-low- and middle-income countries, and less often from high-income countries where monitoring programmes are in place [9, 10, 20, 30].

According to the National Cancer Registry, CC ranks as the fifth most common cancer among Ukrainian women. Currently, Ukraine records 20 cases of CC per 100,000 women [22]. For many years, CC has been proven to be preceded by precancerous lesions-cervical intraepithelial neoplasias (CINs). CINs are most often localised in the cervical epithelial transformation area and are associated with persistent HPV infection.

In Poland (as of 2018), the female population (over 15 years of age) was 17.1 million, with 14.4% of them carrying HPV with normal cytology, which amounted to 2.46 million people. The total number of infected women reached 4.92 million. The estimated number of women with persistent HPV infection who may develop cervical cancer ranges from 246,000 to 492,000 [29]. This cohort of the Polish female population needs timely etiopathogenetic treatment to preserve reproductive function and health, just like women in other countries.

Annually, more than 30 million new cases of mild cervical squamous epithelium dysplasia are diagnosed worldwide, with more than 10 million cases of moderate and severe dysplasia [37]. Regrettably, Ukraine does not record the incidence of cervical precancer in the Cancer Registry. Hence, the data are somewhat contradictory, as the incidence of cervix disorders is recorded based on the published data and ranges from 18 to 43%. Intraepithelial cervical lesions are essentially stages of cervical carcinogenesis. Timely diagnosis and treatment of CIN is a prevention of invasive CC.

To date, numerous studies showed that cervical epithelial dysplasia and cervical cancer develop in the context of CIN [11, 19, 36].

HPV infection is the major and key risk factor for CC. An HPV serotype is detected in 99.7% of CC cases [1]. The epithelial layers of the skin and mucous membranes represent the target cells for this virus. The ultimate result of the transformative effect of HPV is CIN, whose progression leads to CC [3].

Certain types of HPV, high-risk (HR) HPVs, have an increased ability to transform the cervical epithelium with subsequent malignancy. HPV 16 is the most prevalent type globally, found in almost 50% of CC patients, followed by HPV 18 in 15% of CC patients, and HPV 31 and 45 in 10% of CC patients [18, 23, 34].

In Ukraine, HPV 16 is also the most common type, regardless of the patient's age. An epidemiological feature of HPV infection in Ukraine is the high incidence of HPV 53, 31, 33, and 68; HPV 18 ranks only 9th. The risk of HPV persistence and progression of cervical epithelial dysplasia rises when several high viral load HPV types come up simultaneously. Both high- and low-risk HPV types or their combination can be the etiological factor in the development of CIN1 and CIN2 [11, 25].

The national and international literature provides a wealth of information that HPV infection occurs at the level of immature cells of the epithelium of the skin and mucous membranes (basal layer). As the cells move towards the epithelial surface, they differentiate. HPV actively replicates in differentiated cells. The passage of one infected cell from the basal layer to the surface layer can take up to 3 months. This fact determines the need for long-term HPV therapy for at least 3 months [11].

Subsequently, as HPV progresses, a decrease in immune defence and impaired apoptosis play a crucial role.

The immune system's inability to cope with the virus results in long-term HPV persistence in the cervical epithelium. The virus DNA integrates into the epithelial cell DNA. It starts the synthesis of the so-called oncoproteins E_6 and E_7. HPV oncoproteins E_6 and E_7 bind to cellular proteins that suppress the formation of malignant cells (p53 and pRB). This blocks the apoptosis (self-destruction) of the infected cell and blocks the control of proliferation. Consequently, the altered cells divide uncontrollably and intensively, forming a clone of tumour cells.

Importantly, HPV infection of epithelial cells is a prerequisite but insufficient factor for cancer development. The following are also essential to develop irreversible neoplasia: active expression of the E_6 and E_7 genes and high-risk HPV types; induction of metabolic mechanisms for the conversion of estradiol to 16-OH; and induction of multiple chromosomal DNA damage in the infected cell, which completes the degeneration process [41].

Unfortunately, irrespective of the current knowledge of precancerous cervical lesions and their diagnosis and treatment, the incidence of invasive CC is more than 500,000 women a year worldwide, with 274,000 dying from the disease [10]. In Ukraine, 58,419 women were registered with healthcare facilities with CC diagnoses in 2022-2023 [22].

Such statistics compel doctors to improve approaches for optimising the management and treatment of HPV infection and CIN, avoiding overdiagnosis and unnecessary invasive interventions in young women that can interfere with their reproductive function; this is critical in the context of the global demographic crisis, and especially in Ukraine and Poland.

Although proven effective, invasive interventions are traumatic and can disrupt a patient's reproductive plans. At the same time, long-term monitoring and watchful waiting strategies may be dangerous due to the risk of further progression. Therefore, the search for a conservative and safe treatment for HPV infection and CIN1 and CIN2 is still relevant.

Currently, using Ukrainian-made medicinal products PROTEFLAZID® (drops) and PROTEFLAZID® (suppositories) (SMC ECOPHARM LTD) is a promising treatment for HPV-associated CIN1 and CIN2 (mild-to-moderate). The medicinal products have a direct antiviral effect on DNA viruses, which include HPV. The active substances of these antivirals are

the flavonoids apigenin, tricine, and luteolin, obtained from *Herba Deschampsia caespitosa L.* and *Herba Calamagrostis epigeios L.* Flavonoids inhibit DNA polymerases (specific enzymes of papillomaviruses) in cells infected with the virus. These enzymes inhibition leads to the cessation of viral DNA replication, thereby preventing its reproduction.

PROTEFLAZID® (drops) has a specific direct antiviral effect on HPV. This was proven in studies of the specific antiviral activity of PROTEFLAZID® (drops) against HPV in *in vitro* experiments conducted at State Institution „L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases of the National Academy of Medical Sciences of Ukraine" (Kyiv, Ukraine) [24].

An HPV culture model established the specific activity of the active substance of PROTEFLAZID® (drops) in HPV infection at the non-clinical stage of research. The HPV-producing cell culture was obtained by transfection of DNA isolated from infected human cells in suspension cultures of MT 4 cells (suspension culture of human lymphoblastoid cells) and transfected Baby Hamster Kidney fibroblasts (BHK cells). HPV testing was performed by PCR for virus cytopathic action and cytological characteristics such as mitotic index and abnormal mitoses. The HPV isolates were tested with primers for HPV 16, 18, 31, 33, 35, 39, 45, 52, 58, 59, and 67. PROTEFLAZID® (drops) was found to exert a cytopathic effect in the culture of transfected BHK cells, characterised by the formation of transformation foci. *In vitro* experiments established the ability of PROTEFLAZID® (drops) to inhibit HPV reproduction in cell culture by 2 lg ID_{50} [24]. Cytological studies confirmed that following exposure to PROTEFLAZID® (drops), the level of abnormal mitosis in HPV-infected cells was the same as in uninfected cells. PROTEFLAZID® (drops) substantially reduces mitotic activity and the level of abnormal forms of mitosis in HPV-infected cells. These results point to a high degree of inhibition of the proliferative and destructive effects of HPV on cells, which is essential for the clinical efficacy of the specific effect of PROTEFLAZID® against oncogenic HPV. PROTEFLAZID® also inhibits the synthesis of virus-specific enzymes: Thymidine kinase, DNA and RNA polymerases, and reverse transcriptase in virus-infected cells, which is especially important in viral mix infection. The inhibition of cell transformation involves three mechanisms: Inhibition of cell proliferation, induction of cell differentiation, and induction of cell apoptosis. PROTEFLAZID® stimulates apoptosis and, therefore, can inhibit HPV reproduction and cell proliferation. In infected cells, PROTEFLAZID® is capable of inhibiting viral DNA and RNA polymerases. This mechanism was revealed when PROTEFLAZID® (drops) was evaluated in model systems: Transcriptional (DNA-dependent RNA polymerase of bacteriophage T7) and replicative (Taq DNA polymerase) [24].

The above non-clinical studies suggest that PROTEFLAZID® (drops) has specific antiviral activity against DNA-containing HPV.

Furthermore, PROTEFLAZID® (drops) induces the synthesis of endogenous alpha- and gamma-IFNs, which is one mechanism of antiviral action and is confirmed by the detected levels of IFN-alpha and RNase L gene expression. As such, the medicinal product also enhances the body's nonspecific resistance to viral and bacterial infections. PROTEFLAZID® (drops) induces the synthesis of interferons to physiologically active levels without developing refractoriness, whereby immune cells cannot respond to repeated (multiple) inductor administration by producing interferons. Refractoriness leads to the failure of the first line of antiviral protection and, as a result, to developing any infectious complications. Hence, the medicinal product can be used for a long time (vital in treating HPV infection) without risk of immune system depletion. PROTEFLAZID® (drops) stimulates tissue macrophages (increasing their capture, absorption, and digestive capacity), representing an important part of the body's non-specific defence against infectious agents. It can restore the levels of local immunity in both early and late immune responses in the treatment of HPV infection, affecting both early and late immune responses.

PROTEFLAZID® (suppositories) normalises local immunity factors: Secretory IgA, lysozyme, complement component C_3. This results in restoration of the barrier function of the vaginal and cervical mucosa. In addition, the medicinal product helps to resolve dysbiotic disorders of the vaginal microflora, restores the normal vaginal biotope, and accelerates the regeneration of the cervical mucosa epithelium. This is a vital aspect for treating and preventing disease recurrence, given that HPV penetration into the basal cells, where infection occurs, depends on the disruption of the epithelium's integrity and barrier function.

Moreover, PROTEFLAZID® (drops) affects an important mechanism of progression of the HPV infection - the disruption of apoptosis. The medicinal product potentiates the effect of apoptosis-inducing substances and activates caspase 9, thereby promoting the clearance of virus-affected cells and primary prevention of chronic diseases in the context of latent viral infections.

PROTEFLAZID® (drops and suppositories) has a direct antiviral effect on HPV and mechanisms of influence on key processes of HPV progression (which can lead to tumour transformation of the cervical epithelium), the possibility of prolonged use (three months or more) with no development of immune system refractoriness allows us to consider the use of PROTEFLAZID® (drops and suppositories) in the treatment of patients with diagnosed HPV infection complicated by mild-to-moderate cervical epithelial neoplasia as an etiopathogenetic treatment of HPV infection and secondary prevention of CC.

Currently, PROTEFLAZID® is available in two pharmaceutical forms: Oral drops and vaginal suppositories. The availability of two forms provides for concurrent systemic and topical use and improves the effectiveness and efficacy of HPV therapy.

AIM

To review the specific activity of PROTEFLAZID® Drops and Suppositories at the stage of non-clinical study, and to assess the efficacy and safety of its use in clinical practice in HPV-associated female reproductive system diseases.

MATERIALS AND METHODS

Analysis of scientific publications on the treatment of palilomavirus infection with PROTEFLAZID® in women over the past decade.

REVIEW AND DISCUSSION

In 2015, Ukrainian scientists published a systematic review of the literature accompanying the analysis of the first scientific publications on the experience gained to date in the use of medicinal products PROTEFLAZID® in clinical settings in the treatment of HPV-associated diseases[12]. The review of the literature on non-clinical studies revealed that the active substance of PROTEFLAZID® (drops) had direct antiviral action against DNA-containing human papillomaviruses. In addition, PROTEFLAZID® (drops) induces the synthesis of alpha- and gamma-interferons, which contributes significantly to the medicinal product's indirect antiviral effect.

A systematic review of the literature on the clinical use of PROTEFLAZID® (drops) confirmed the medicinal product's antiviral activity against HPV. This is consistent with the results of non-clinical studies and confirms the product's efficacy in HPV-associated diseases in women (including those in pregnancy) and men of reproductive potential. A strong clinical effect was achieved in more than 1500 patients treated with PROTEFLAZID® (drops). The absence of adverse events confirmed the safety. The information is based on the results of independent clinical observations with coincidental positive effects of the treatment.

Summing up, the authors emphasised that the studies prove that the active substance of PROTEFLAZID® has a polypharmacological effect, in particular: Antiviral, immunocorrective, antioxidant, and apoptosis-modulating, which contributes to a stable therapeutic effect, HPV eradication, elimination of vaginal microflora dysbiosis, restoration of normal vaginal flora, reduction of the incidence of recurrence and HPV infection, as well as normalisation of the cervical epithelium, reduction of the proliferative activity of neoplasms, which allows the use of organ-preserving treatment in the reproductive population as a guarantee of future parenthood [12].

PROTEFLAZID® (vaginal tampons impregnated with drops) as a topical, intravaginal treatment normalises cytology and promotes the transition of cervical intraepithelial neoplasia of class CIN2 (moderate dysplasia) to class CIN1 (mild dysplasia), reducing the severity of signs of cervical epithelial dysplasia and contributing to a significant reduction in HPV DNA viral load compared to baseline. PROTEFLAZID® (vaginal tampons impregnated with drops) in the treatment of HPV-associated diseases helps to normalise clinical and laboratory parameters and eliminate HPV by more than 3.6 lg in 80% of patients, as confirmed by PCR.

The efficacy of PROTEFLAZID® (vaginal tampons impregnated with drops) was proven at the stage of preconception preparation of infected women for pregnancy and reduction of the frequency of perinatal complications of the gestational period. No significant adverse events were reported during long-term use of the medicinal product in pregnant women [12].

In a clinical trial [7] on the use of PROTEFLAZID® (drops) and PROTEFLAZID® (vaginal tampons impregnated with drops) in the treatment of genital HPV infection in women with systemic and vaginal administration to treat benign and precancerous cervical lesions, the authors concluded the efficacy of PROTEFLAZID® (vaginal tampons impregnated with drops) and PROTEFLAZID® (drops). The standard of care is inferior in terms of virostatic and anti-relapse effects to combination treatment with PROTEFLAZID®. Given that PROTEFLAZID® reduces the viral load in HPV infection by 3.0 lg D_{50} and increases the number of antigen-presenting cells, using these medicinal products as part of combination therapy in all patients with HPV infection is clearly warranted.

A study on the comparative efficacy and safety of PROTEFLAZID® Suppositories and Drops (vaginal tampons impregnated with drops) in patients with HPV-associated cervical epithelial dysplasia [14] showed that PROTEFLAZID® (suppositories) is highly effective and not inferior in therapeutic efficacy to PROTEFLAZID® (vaginal tampons impregnated with drops) when used in patients with HPV-associated cervical epithelial dysplasia (CED). After completing the treatment course with PROTEFLAZID® (suppositories), cytology or transition of cervical intraepithelial neoplasia was normalised from CIN2 to CIN1. Following treatment with PROTEFLAZID® (vaginal tampons impregnated with drops) and PROTEFLAZID® (suppositories), an 8-week follow-up confirmed a decrease in the severity of the signs of cervical intraepithelial neoplasia (leukoplakia, punctation, acetoblastic epithelium, mosaic, atypical transformation zone); improvement of cytological findings (decrease in leukocytosis, coilocytosis, dyskeratocytes); a significant increase in the level of local immunity (no significant differences between the groups in local immunity (sIgA, lysozyme and C3 complement component) at all evaluation time points); a significant decrease in the HPV DNA viral load, indicating a delayed antiviral effect of the medicinal products (no significant differences between the groups in the level of HPV viral load at all evaluation time points). Both pharmaceutical forms PROTEFLAZID® Suppositories and Drops (in the form of vaginal tampons impregnated with drops) proved to be well tolerated, but the suppositories have a more convenient dosing regimen, unlike the drops, which, at the same dosing frequency, required time to prepare the appropriate dilution and impregnate vaginal tampons. The authors recommended PROTEFLAZID® (suppositories) as an effective and safe antiviral treatment for HPV-associated female reproductive system diseases [14].

Given the high efficacy and safety of PROTEFLAZID® (drops and suppositories) in the treatment of HPV, the authors recommended using the medicinal products in routine clinical practice to treat women of reproductive potential [12].

A team of authors [13] conducted a meta-analysis of the results of long-term clinical trials to assess the efficacy of PROTEFLAZID® (drops and suppositories) in the treatment of HPV infection (the results of treatment in more than 1000

patients were analysed). The meta-analysis confirmed the high efficacy of PROTEFLAZID® (drops and suppositories) in the treatment of patients with HPV-associated urogenital and cervix disorders. The meta-analysis revealed the following: The likelihood of recurrence of condylomatous manifestations in the intervention group (treated with PROTEFLAZID® (drops and suppositories)) is 5.5 times lower than in the control group (not treated with PROTEFLAZID®); the likelihood of HPV detection (by PCR) in the intervention group is 6.7 times lower than in the control group; the likelihood of resolution (significant reduction) of HPV replication as determined by PCR in the intervention group is 4.7 times higher than in the control group. The medicinal products PROTEFLAZID® (drops and suppositories) promote the clearance of HPV from the body, preventing the recurrence of the disease and reducing the risk of virus-induced neoplastic lesions. PROTEFLAZID® (drops and suppositories) is recommended as an effective antiviral medication to treat HPV-associated cervix and urogenital disorders as an etiopathogenetic treatment in the acute period, during recovery and in persistent HPV infection [13].

In a study comparing the efficacy of different regimens of PROTEFLAZID® (drops and suppositories) in the treatment of mild-to-moderate cervical intraepithelial neoplasia associated with genital HPV infection, the team of authors found that treatment with PROTEFLAZID® (drops and suppositories) for HPV-associated CIN1 and CIN1 is etiologically and pathogenetically justified, given the direct antiviral effect of the medicinal product on HPV and the mechanisms of action on the transformation of cells affected by the virus [11]. Systemic use of PROTEFLAZID® (oral drops) to treat mild-to-moderate neoplasia improved the cervical epithelium in 60% of cases and eradicated HPV in 73.3% of patients. Topical use of PROTEFLAZID® (suppositories) in CIN1 and CIN2 normalised cytology or led to the transition of CIN2 to CIN1 in 63.3% of women; no HPV DNA was detected after treatment in 76.7% of cases. At the same time, 3 months of concurrent topical and systemic use of PROTEFLAZID® (drops and suppositories) to treat HPV-associated CIN1 and CIN2 resulted in regression of CIN1 to normal and CIN2 to CIN1 in 83.3% of patients; 90.0% of patients did not have HPV DNA detected after treatment. Clearly, this regimen of concurrent topical and systemic use of two pharmaceutical forms of PROTEFLAZID® (oral drops) and PROTEFLAZID® (suppositories, vaginal) provides the highest therapeutic efficacy [11].

The authors recommended PROTEFLAZID® (suppositories) and PROTEFLAZID® (drops) to be introduced into clinical practice as a safe and efficacious treatment for HPV-associated CIN1 and CIN2 as monotherapy, especially in the regimen of concurrent systemic and topical use of PROTEFLAZID® (drops + suppositories), ensuring the highest therapeutic efficacy. This regimen of concurrent use of two pharmaceutical forms of PROTEFLAZID® (drops and suppositories) is a promising approach to the treatment of HPV infection and CIN 1-2 in women of reproductive potential, as it helps to prevent cervical damage, early and late complications after invasive interventions and preserve female reproductive function [11].

In a study of the effect of PROTEFLAZID® (suppositories) on local immunity in disorders associated with human papillomaviruses, herpesviruses, or mixed genitourinary infections, a team of authors [31] revealed that PROTEFLAZID® (suppositories), when used to treat sexually transmitted diseases, significantly improves such local immunity parameters as sIgA, lysozyme, and complement component C_3 in cervical mucus. This improvement is sustained and persists for a long time after treatment. Furthermore, the medicinal product contributes to the correction of deregulated natural defence factors during exacerbation of mixed infections, when activation of the complement component C_3 may occur due to decreased sIgA and lysozyme levels. Following treatment with PROTEFLAZID® (suppositories), all three components of the natural defence response increased, and the ratios between them reached optimal values, which can provide the most significant anti-infective protection [31].

The relationship between macro- and microorganisms in vaginal homeostasis is complex. It is difficult to imagine that a single protective factor can prevent the onset and development of infection in the host. Only a complex of protective factors inherent in the body and in an active state can actually resist infection. In other words, 'aggregation' (in this case, combining components into a single system) and mobilisation of defence factors can actually resist the local spread of infection. This can explain the positive effect of PROTEFLAZID® (suppositories) when used topically to treat sexually transmitted diseases, when after a course of treatment with the medicinal product, the level of such key defence factors as sIgA, lysozyme, and complement component C_3 in cervical mucus increases significantly. Thus, the medicinal products PROTEFLAZID® (suppositories or vaginal tampons impregnated with drops) significantly and consistently improve the content and ratio of such major local immunity factors as sIgA, lysozyme, and complement component C_3, thus enhancing the anti-infective protection of cervical mucus in the female reproductive system as a whole, in the treatment of sexually transmitted diseases and infections caused by human papillomaviruses, herpes viruses, and mixed urogenital infections (herpes viruses + chlamydia) [31].

A study [5] on the clinical efficacy of PROTEFLAZID® Vaginal Suppositories for topical use in the treatment of HPV-associated mild-to-moderate cervical intraepithelial neoplasia showed that a tricyclic monotherapy (14 days for 3 months) has a positive effect on the cervix, according to the results of the PAP test based on liquid cytology – a decrease in the detection of low-grade squamous intraepithelial lesions (LSIS) from 80% to 8%, normalisation in 82% of cases; colonoscopic findings – 82% of women had normal findings after treatment; disappearance of proliferation markers p16 and Ki-67 in all cases; significant reduction of viral load by 1.8 times; morphologically confirmed regression of cervical intraepithelial neoplasia in 96% of cases. A positive effect of suppositories on the vaginal flora

was also reported, as evidenced by a 2.16-fold decrease in the number of leukocytes in the field of view and a 5.6-fold increase in the frequency of lactobacilli detection. Additionally, this treatment was well tolerated by patients, convenient, provided in outpatient settings, painless, and had virtually no side effects [5].

In a clinical paper dedicated to the study of ways to reduce diagnostic and therapeutic intervention in HPV-infected patients of reproductive potential found that after 3 months of treatment with PROTEFLAZID® systemically (drops) and topically (suppositories), regression of CIN was observed in 93% of patients [2]. A reduction in viral load of more than 2 Lg HPV/10^5 was recorded in all cases, which is a marker of the effectiveness of antiviral therapy. At 6 months post-treatment, 84% of patients and at 9 months, 88% of patients had complete HPV eradication or reduced viral load to clinically insignificant levels. The patients tolerated the therapy well, and mild vaginal itching in the first days of suppository administration did not require discontinuation. After treatment with PROTEFLAZID® (drops + suppositories), 93% of women did not require invasive interventions, which is essential given their reproductive plans.

Based on the data obtained, the authors recommended a treatment regimen that includes concurrent use of PROTEFLAZID® (suppositories for topical use) and PROTEFLAZID® (drop for systemic use) as an efficacious and safe way to treat patients with HPV-associated cervical intraepithelial neoplasia CIN1 and CIN2. The used treatment regimen enables practitioners to reduce unnecessary medical and invasive interventions in the treatment of patients with HPV-associated disorders. Successful treatment of CIN is the cornerstone of cervical cancer prevention in women of reproductive potential [2].

In the National Consensus (Ukraine) on the Management of HPV-Associated Cervical Intraepithelial Neoplasia [8], the leading experts of Ukraine stated that the most perspective direction in the treatment and secondary prevention of HPV infection is the use of direct antivirals (which include the active substance of PROTEFLAZID®), which are also immunomodulators, activate apoptosis and have an antiproliferative effect on cells [8].

The concurrent comprehensive systemic and topical use of the medicinal products PROTEFLAZID® can be used to successfully treat CIN1 and CIN2 without surgery. The use of medicinal products with direct antiviral action, such as PROTEFLAZID® (drops and suppositories), is necessary to provide anti-relapse therapy and help restore and strengthen the immune system, which is vital in HPV treatment.

An important consideration is the treatment of cervical intraepithelial neoplasia in pregnancy. However, the treatment of pregnant women has certain difficulties due to the impossibility of using many drug classes that are not approved or recommended for use in pregnant women. Yet, the medicinal products that combine direct antiviral action and immunomodulatory activity and are approved for use during gestation, such as PROTEFLAZID® (drops and suppositories), can not only improve the effectiveness of treatment of certain virus-induced disorders, but also reduce the incidence of obstetric and perinatal complications of pregnancy in such women [28].

A study on the results of clinical and molecular histological evaluations of the efficacy of the medicinal product PROTEFLAZID® is greatly relevant and is encouraging for further progress in the field of HPV infection treatment in women with cervical intraepithelial neoplasia [21]. His clinical and molecular morphological evaluations proved that concurrent use of PROTEFLAZID® (drops) and PROTEFLAZID® (suppositories) for 3 months in HPV-infected women without signs of cervical morphological damage contributed to HPV eradication in 86.7% of cases and in women with CIN1 caused by HPV – in 73.3% of cases. In addition, treatment contributed to the regression of cervical dysplasia in 76.7% of women. Treatment of HPV infection with PROTEFLAZID® in women with CIN1 leads to a steady regression of dysplastic lesions of the cervical epithelium, as all Pap tests and HPV tests were negative during 24 months of post-treatment follow-up in the treated women [21].

The results clearly indicate the need for antiviral treatment in women at the early stage of HPV infection, when cytology specimens show coilocytosis, i.e. before HPV can cause CIN1 or more severe dysplastic cervical lesions. Such treatment can be considered primary prevention of cervical cancer, as women without HPV infection were shown to have a shallow risk of developing cervical cancer.

Currently, the medicinal products PROTEFLAZID® (drops and suppositories) are among the most effective treatment options for HPV infection, used and recommended by healthcare professionals for HPV eradication in women with both cervical intraepithelial neoplasia and a positive HPV test with a normal Pap test [8].

Note: As of 30/09/2023, the cumulative sales volume of PROTEFLAZID® (drops, since marketing authorisation) and the number of sold vials (since 2006) in Ukraine and abroad (in vials) amounted to 5,629,300 vials. The cumulative sales volume of PROTEFLAZID® (suppositories) since its launch in Ukraine (2016) and abroad up to 01/03/2023 amounted to 414,306 packs No. 10. The cumulative pooled pharmacovigilance monitoring data from the local regulatory agency and available literature data indicate few reports of non-serious adverse events associated with both PROTEFLAZID® (drops) and PROTEFLAZID® (suppositories).

Given the current martial law regime and the challenging situation with clinical trials in Ukraine, the Company would be grateful to a foreign investor who would agree and/or directly participate in the further expansion of the trials mentioned above of PROTEFLAZID® (suppositories) and PROTEFLAZID® (drops) to treat HPV-associated disorders and prevent cervical cancer in women of reproductive potential in Poland or one of the European countries.

A clinical trial to investigate the combined effect of the investigational product PROTEFLAZID® (capsules; Phase II clinical trials of this pharmaceutical form were successfully completed in Ukraine) with systemic and topical use of PROTEFLAZID® (suppositories) to treat HPV-associated disorders in women of reproductive potential in one of the European countries could be of particular interest to potential European investors.

The active substance of PROTEFLAZID is of natural origin, highly active, and non-toxic (Category 5 toxicity). At present, Ecopharm manufactures the amount of the Active Pharmaceutical Ingredient that would be sufficient to treat HPV-associated disorders in women for the prevention of cervical cancer in one of the European countries, such as Poland or Germany.

Upon completion of clinical trials and marketing authorisation of the PROTEFLAZID® pharmaceutical forms (drops and suppositories) in a specific country, Ecopharm will be able to fully address the issue of treatment of HPV infection and cervical dysplasia (caused by HPV infection, CIN1 and CIN2), providing timely and effective prevention of cervical cancer in women of reproductive potential, which, in turn, is critical for non-invasive therapy and overcoming the challenges of the current demographic crisis.

CONCLUSIONS

PROTEFLAZID® (drops and suppositories) are safe and effective etiopathogenetic medications for systemic and local treatment of HPV-associated disorders in women. The medicinal products show potential for the prevention of cervical cancer, as they allow for organ-preserving treatment in women of reproductive potential as a pledge of future motherhood.

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CONFLICT OF INTEREST
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