Novel Vaginal Gel Use for Vulvovaginal Atrophy in Women with Breast Cancer

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Vulvovaginal atrophy (VVA) is a common condition in women associated with decreased estrogen levels in vaginal tissues. It often occurs during menopause or as a side effect of breast cancer therapies. Currently, the most effective treatment for VVA is topical estrogen, but its use in breast cancer patients is controversial due to systemic absorption concerns. This has created a need for safe alternatives such as Ospemifene.

Ospemifene is an FDA-approved oral selective estrogen receptor modulator (SERM) for treating VVA symptoms. However, its limited bioavailability, delayed relief, and potential risks hinder its efficacy. To overcome these challenges, we propose developing a vaginal micro-sponge gel with Ospemifene as the active agent, aiming to provide a safe and effective topical therapy for VVA in breast cancer patients.

Our first aim is to develop a unique gel formulation using Ospemifene, hyaluronic acid, glycerin, and a low-dose progestin to prevent endometrial cancer. We will evaluate the formulation's compatibility and stability under different vaginal conditions. In the second aim, we will assess the effectiveness of Ospemifene using an ex-vivo tissue model, evaluating its ability to promote vaginal tissue growth and regeneration. We will also examine its safety by assessing its impact on cervical-vaginal cell lines and lactobacillus viability. The third aim involves testing the gel on a VVA rat model to evaluate its efficacy and safety. By generating comprehensive data, we aim to establish a novel and safe route for Ospemifene administration, providing a viable alternative to topical estrogen for the treatment of VVA. This research has significant implications for

improving the quality of life for women with a history of breast cancer. By offering a safe and effective therapeutic option, it addresses the limitations of current treatments and fills a crucial gap in VVA management.

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1.0 Specific Aims

Vulvovaginal atrophy (VVA) is a common condition in females, caused by a decrease in estrogen levels in vaginal tissues. VVA may causes vaginal dryness, irritation, soreness, dyspareunia, and urinary tract infections that can greatly decrease patient quality of life. Currently, more than 2 million women in the United States have a history of breast cancer, many of whom are on chemotherapy. Chemotherapeutic agents induce a hypoestrogenic status that commonly results in VVA. The prevalence of VVA in breast cancer patients ranges from 23-61% (depending on menopausal status), which highlights the importance of providing an effective therapy. Currently, the standard of care for treating VVA is the topical application of estrogen. However, the use of estrogen in breast cancer patients, even in small topical doses, is controversial because it may enhance tumor growth and proliferation. Therefore, the use of estrogen is not recommended in this population of women, and this generates a need for safe, non-hormonal therapy options for VVA in women diagnosed with breast cancer. One alternative to topical estrogen is Ospemifene. Ospemifene is an oral selective estrogen receptor modulator (SERM) that is FDA approved for the treatment of symptoms associated with VVA in menopausal women. Ospemifene use is accompanied with a multitude of drawbacks which include poor bioavailability and tissue distribution, delayed onset of action and symptom relief, increased risk of endometrial cancer, and adverse drug reactions such as nausea and vomiting. These limitations are already a concern for patients undergoing breast cancer treatment and are associated with a discontinuation rate of 15% for Ospemifene. Moreover, Ospemifene has never been indicated for use in breast cancer patients. Therefore, a topical formulation of Ospemifene that addresses these concerns is required. Micro-sponges are a novel drug delivery system that are designed for delivering drugs efficiently in lower

doses with enhanced stability, improved drug release profile and minimized side effects**.** In this proposal, we hypothesize that the Ospemifene micro-sponge gel will 1) reduce dosage while maintaining therapeutic efficacy and 2) minimize side effects by prolonging in-vivo retention and limiting systemic exposure. We plan to test our hypothesis with the following three aims:

1.1 Aim 1 will develop and characterize a novel Ospemifene micro-sponge-based gel.

Ospemifene micro-sponges will be developed using the quasi-emulsion method using Eudragit RS100.To further enhance its efficacy and safety, hyaluronic acid and glycerin will be used as lubricants/moisturizers to expand on the gel's acute effectiveness. Progesterone will be incorporated into the Carbopol gel to minimize the proliferative effects of Ospemifene on the endometrium. From the reported data, a drug (Ospemifene) to excipient ratio is selected for the gel and further optimized according to release rate, drug content, pH, viscosity, and bio adhesion. Finally, in this aim, the stability of the formulation will be evaluated under varying vaginal conditions including pH, enzymes, oxidation, and temperature and in the presence of the other excipients. The formulation that meets quality criteria will be used for subsequent studies.

1.2 Aim 2 will investigate the gel formulation for ex-vivo efficacy and safety.

In this aim, the gel's efficacy will be demonstrated directly on an ex-vivo tissue model to assess its effect on vaginal tissue growth and regeneration under conditions that mimic in-vivo conditions. Additionally, different studies will be conducted to demonstrate its safety, including

cellular toxicity studies on CaSki and VK2 cell lines, using Luminescent cell titer glow assay, Lactobacillus compatibility/viability by performing a susceptibility test using the disk diffusion method, and vaginal tissue viability by looking at tissue integrity, histology, and morphology using different stains. We expect our formulation to be safe and stable while allowing the regeneration of vaginal tissue.

1.3 Aim 3 will evaluate the safety and efficacy of the Ospemifene micro-sponge-based gel on a VVA rat model.

In this aim, we plan on using a VVA rat model to assess the gels efficacy by observing changes in vaginal epithelial weight & thickness and pH, progesterone /estrogen receptor ratio, glycogen, elastin, and drug levels over a period of 12 weeks. Additionally, to ensure safety of the gel, we aim to examine uterine tissue weight, plasma and tissue drug levels, and vaginal histological and morphological changes. The data generated will be compared to reported data on the oral formulation for safety, efficacy, and local/systemic drug levels. We expect the gel to be as effective and safe as the oral formulation with higher local drug levels and lower systemic drug levels.

Collectively, the data generated from this study will aid us in establishing a novel route of administration for a Ospemifene based gel that would be used to treat VVA in breast cancer patients. At the conclusion of our planned work, an Ospemifene vaginal gel that is effective, safe, and stable will be generated and positioned to be further developed into a clinical trial candidate.

2.0 Research Strategy:

2.1 Background

2.1.1 VVA is common, bothersome condition that directly impacts the quality of life of affected women.

VVA is caused by decreased estrogen levels in vaginal tissues. This leads to thinning of the epithelial lining, which subsequently results in dynamic and drastic changes in the vaginal cavity..(1) VVA is commonly associated with conditions where levels of estrogen are significantly reduced, such as menopause and breast cancer therapy. Currently, more than 2 million women in the United States every year have a history of breast cancer, many of whom are on therapy.(2) Breast cancer therapeutics (surgical, endocrine and chemotherapeutic agents) can cause estrogen deprivation and thus cause or exacerbate VVA. The prevalence of VVA in women with breast cancer is 23% in premenopausal and 61% in postmenopausal women.(3) Typically, women can present with a wide range of undesirable symptoms that are frequently underreported. These symptoms include vaginal dryness, irritation, soreness, dyspareunia, and urinary tract problems.(4) Additionally, VVA can disrupt the normal function of lactobacillus potentially causing an increase in vaginal pH, which in turn increases the risk of opportunistic infections and sexually transmitted diseases. Importantly, these symptoms can negatively affect women's quality of life (QoL).(5-7) It has been reported that women with frequent and severe VVA symptoms had impaired QoL and reduced sexual function.(8) Therefore, providing safe and effective methods of treatment is paramount.

2.1.2 Estrogen-based therapies are effective, but their use is contraindicated for women diagnosed with breast cancer.

Currently, the first line treatment for VVA is low dose vaginal estrogen. Estrogen is available in multiple dosage forms such as vaginal creams, tablets, and rings. They have an advantage of being cheap and widely available.(1) A concern with the use of vaginal estrogen in breast cancer patients is systemic absorption. Several studies have shown that plasma estrogen levels increase compared to baseline after vaginal administration.(9-11) Elevated systemic levels of estrogen may exacerbate cancer proliferation and counteract the therapeutic effects of chemotherapy. Based on these findings, the use of vaginal estrogen in women diagnosed with breast cancer is discouraged. Alternatively, non-hormonal therapies utilizing moisturizers and lubricants are recommended for women diagnosed with breast cancer and suffering from VVA. Commonly used lubricants and moisturizers include active ingredients such as polycarbophil, glycerin, dehydroepiandrosterone, and hyaluronic acid.(12, 13) They work by attaching to the epithelial surface and adsorbing water, which causes brief symptomatic relief. Compared to estrogen, lubricants and moisturizers have a shorter duration of action and are considered less effective.

2.1.3 Oral Ospemifene is an effective non-hormonal therapy for VVA.

Ospemifene is an oral SERM that is currently FDA approved for the treatment of VVA in postmenopausal women.(14).Ospemifene exerts an estrogenic action on the vaginal epithelium with minimal effects on the endometrium and neutral effects on the breast. Ospemifene is highly effective for the treatment of VVA (comparable to estrogen). Improvement in VVA symptoms is

observed within 12 weeks of use with no major side effects.(15-18) The use of oral Ospemifene is associated with drawbacks. First, Ospemifene absorption and bioavailability relies on food, and breast cancer patients on therapy usually present with a decrease in appetite.(19) Moreover, Ospemifene has a delayed onset of action and symptomatic relief takes a while to be observed. Finally, all SERMs are associated with an increased risk of endometrial cancer and nausea/vomiting, which is already a concern for many breast cancer patients. Despite the presence of data that suggests that it is safe for use in breast cancer patients, Ospemifene is not yet approved for use in that group of patients.(20) All these factors contributed to a discontinuation rate of 15%, which highlights the importance of finding an alternative route that addresses the associated drawbacks. (21)

2.1.4 Micro-sponge-based gels are novel drug delivery systems that can be used to improve therapeutic outcomes.

Micro-sponges are polymeric delivery systems composed of porous microspheres (5-300 μm size range). They are sponge-like spherical particles with a large porous surface. Given the large size of the micro- sponges, they are retained in mucosal pockets with minimal absorption. This allows for sustained release of the active agent. Additionally, micro-sponges are able to entrap and load a greater amount of active ingredient compared to liposomes and similar novel drug delivery systems (50-60% greater drug loading). Additionally, the presence of a large number of sponges like channels allows for sustained release by controlling the rate of diffusion of the drug molecule. (22). Micro-sponges offer high chemical stability due to inertness of the encapsulating excipient with many different active pharmaceutical ingredients. Moreover, micro-sponges are stable over a pH range of 1-11 and temperatures up to 130°C.

Furthermore, the structure and pore size of micro- sponges are too small (<1 μm) to permit bacterial infiltration and microbial growth. The methods available to produce micro-sponges are either with liquid–liquid suspension polymerization or quasi-emulsion method which are deemed efficient and reproductible. The quasi-emulsion method produces microspheres by using a hydrophobic polymer to entrap the drug. Once sufficient time has passed, an emulsifier is added to allow organic solvents to diffuse out of the mixture, which mediates the formulation of the micro-sponges. Using these methods, many different therapeutic agents have been prepared as micro-sponge dosage forms intended for topical delivery. Examples include: Anti-inflammatory agent diclofenac diethylamine for arthritis; fluconazole, miconazole, and sertaconazole for fungal infections; nebivolol for wound healing.(23-27) Given their ability to provide an excellent controlled release profile, micro-sponges are less likely to cause mucosal irritation. Therefore, their use for VVA is ideal as they would 1) reduce the likelihood of irritation and side effects due to the controlled behaviors of drug release and inertness of excipients used 2) improve drug release, duration of action, and stability 3) lower the dose required by increasing drug localization and overcoming barriers that affect drug bioavailability and distribution.

2.1.5 Gels are the ideal vaginal formulation for treating VVA.

Multiple vaginal dosage forms exist for treating various local diseases. Gels can be designed to improve wetting efficacy and in cooperated with excipients that can cause surface hydration which ultimately help soothe and alleviate the symptoms of VVA. For many vaginal conditions, gels are the preferred dosage form due to ease of use and availability.(28, 29). Gels are known to have a short duration of action and require frequent application. Gels can function as a vehicle for active drug agents and novel drug delivery systems such as liposomes,

microcapsules, and micro-sponges. The use of a micro- sponge-based drug delivery system aids the gel in providing a sustained release formulation and therefore reduces the frequency of application. The micro-sponges are retained inside the gel due to their hydrophobic nature and large particle size; this slows down their release and upon release they are retained in pockets present on the vaginal epithelium.

2.2 Innnovation

2.2.1 This will be the first study where a SERM vaginal gel will be developed as micro-sponge dosage form and used for the treatment of VVA in combination with other therapeutic ingredients.

A vaginal formulation of Ospemifene has never been developed before and has never been used in combination with lubricants/moisturizers. Given the advantages of a micro-sponge dosage form, an Ospemifene based micro- sponge gel with the addition of lubricants/moisturizers will improve therapeutic outcomes. Lubricants/moisturizers can provide symptomatic relief in a short period of time, giving Ospemifene time to exert its full therapeutic action. The addition of progesterone will address any concerns related to SERM-induced endometrial cancer. Ultimately, the goal of this study is to provide women diagnosed breast cancer a product that is safe, effective, and can treat both short-term and long-term symptoms of VVA**.**

2.3 Preliminary Data

2.3.1 Hypothetical mechanism of action of the Ospemifene micro-sponge gel.

Carbopol is a polyacrylic acid (PAA) polymer that releases and accepts protons in basic and acidic pHs respectively. The pH change facilities the process of osmosis into the gel. Point (1) in the figure shows the water influx due to the presence of highly ionized species which interacts via dipole-polar and hydrogen bond interactions. In turn (Point 2), PAA swells due to the electrostatic repulsion of the negatively charged groups, releasing the drug molecules to the environment. Additionally, Carbopol residence time is increased due to its interaction with mucin that occurs by four mechanisms viz. electrostatic interaction, hydrogen bonding, hydrophobic interaction and inter diffusion.

Once Hyaluronic acid (HA)(Point 3) is released from the gel and it interacts with CD44 receptors present on the surface of vaginal epithelial tissue. Moreover, HA binds to elements in the extracellular matrix (ECM), which in turn traps water between the ECM and the tissue. In addition to the gels soothing effect, the effect of HA causes acute symptomatic relief of VVA by hydrating the surrounding vaginal tissues. The release of progesterone (Point 4) takes on a zero-order release, mainly through diffusion out of the gel after swelling. The primary site of action for progesterone is the vagina (thickening of mucus) and the endometrium (thinning of the endometrial tissue). (Point 5) Ospemifene molecules are entrapped in the micro-sponges and are released via two mechanisms (passive diffusion through the pores or erosion of the micro-sponge polymer) until the gel is saturated with Ospemifene. Once the gel is administered Ospemifene moves out of the gel and equilibrium has been reestablished by the release of the drug into the gel once again. Ospemifene interacts with Progesterone and HA through Van der Waal interactions

and hydrogen bonding that are easily disrupted due to the presence of an abundant amount of water molecule that surrounds and shells every drug/excipient particle. Upon release, Ospemifene interacts with the estrogen receptors present on the surface of the vaginal epithelium (similar affinity towards alpha and beta subtypes). Once it binds it causes dimerization of the estrogen receptors which recruits and interact with various co-activators and corepressors, the complex then moves in into the nucleus and start or inhibit the process of transcription. Depending on the tissue, Ospemifene can cause an agonist effect in the vagina by binding to ER and stimulating the release of co-activators which facilitate the process of transcription. In contrast, when Ospemifene binds to the breast tissue it predominately causes the recruitment of corepressors which produce an antagonist effect. Ultimately, Ospemifene will cause the proliferation of vaginal epithelial tissue without inducing any changes in the breast tissue.

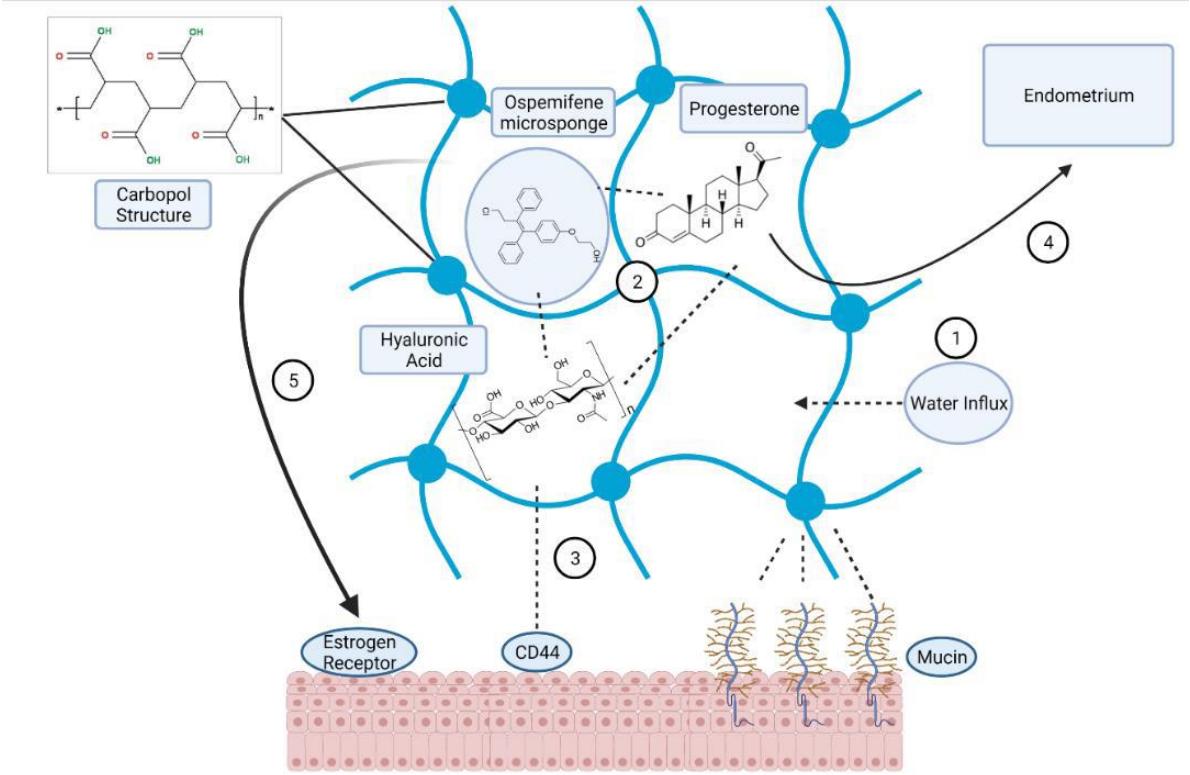

Figure 1: Chronical schematic overview of the different components of the Ospemifene micro- sponge gel

2.3.2 Quantitative analysis of Ospemifene using high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS).

The HPLC quantification of Ospemifene will be carried out using a validated HPLC method.(30)The HPLC is equipped with a PDA detector as previously described in the literature in accordance with ICH guidelines. A reverse phase column (Agilent (5µm,4.6x150mm) maintained at 25°C will be used, flowing in a rate of 0.7 ml/min with a run time of 10 minutes. The flow is isocratic with a Mobile phase A to B (Water: Acetonitrile 40:60%). Sample injection volume is 10 µl and the wavelength for maximum detection is set at 274 nm.(31) For LC-MS quantification, the LC part is carried out using an Agilent Zorbax, XDB-Phenyl, 4.6 × 75 mm, 3.5 µm column, with a column temperature of 40 C. The mobile phase consisted of methanol and

ammonium formate in the ratio 90:10 (v/v), which is filtered through a 0.45 μm membrane filter, degassed, and delivered at a flow rate of 0.9 ml/min. Ospemifene-d4 will be used as an internal standard (IS). The MS portion was set on positive ion mode. The ion spray voltage is set at 5500 V. The source parameters viz. the nebulizer gas, curtain gas, auxiliary gas and collision gas were set at 40, 25, 40 and 7 psi, respectively. The compound parameters, viz. the declustering potential, collision energy, entrance potential and collision cell exit potential, were 90, 28, 10 and 18 V for ospemifene and the IS. The ions detection was carried out in the multiple-reaction monitoring mode (MRM), by monitoring the transition pairs of m/z 379.2 precursor ions to m/z 205.2 for ospemifene, m/z 383.2 precursor ions to m/z 205.2 for the IS. Quadrupoles Q1 and Q3 were set on unit resolution.(32)

2.3.3 Quantitative analysis of Progesterone using HPLC.

Progesterone is quantified using a previously established validated HPLC method. The HPLC is equipped with a PDA detector set at a wavelength of detection at 241 nm. Mobile phase A consists of phosphate buffer: Acetonitrile 90:10 v/v ratio. Mobile phase B consists of Acetonitrile: Water 90:10 v/v ratio. The flow is gradient set at 1 ml/min with a run time of 30 minutes (Gradient set at A:B – 55/45 at 0-2 minutes, 50:50 2-16.5 minutes, 5:95 16.5-24 minutes, 55:45 24-30 minutes). Column used is Eclipse XDB C8 (150 x 4.6 x 5 um) set at 30 C. Injection volume is 10 ul and diluent used is 80% Acetonitrile in water.

2.3.4 Developing micro-sponges using the Quasi-Emulsion method.

As previously reported, micro-sponges are manufactured using the Quasi-emulsion

method. The initial steps start with mixing the drug in combination with Eudragit RS 100, which is used to extend and sustain the release of the drug. Ideally, both the drug and excipient should dissolve in a suitable solvent, which is usually an organic solvent. In the case of Miconazole, a drug with similar properties to Ospemifene, the best solvent to be used is ethyl alcohol dissolved under ultrasonication for 10 minutes at 35°C. Once the Ospemifene and Eudragit yield a homogenous mixture, it is then mixed with an polyvinyl alcohol (PVA) and water. Following stirring at 500 RPM for 2 hours, the solution is filtered, and the micro-sponges are picked out. The micro-sponges were air-heated in a 40°C oven for 12 hours. See Figure (2) for a schematic representing the quasi-emulsion method. The amount of drug to polymer ratio, the volume of PVA and organic solvent can all influence production yield and entrapment efficiency. Once the micro-sponges are made, the production yield and entrapment efficiency can be calculated, as the initial weight of drug used is known. The micro-sponges are dissolved in ethanol, sonicated for 20 minutes and the content is analyzed using an appropriate quantitative method.(33-39) The final micro-sponge drug delivery system is highly porous, with adjustable particle size that is able to retain an excellent amount of drug loading (See Figure 3).

2.3.5 Vaginal Gel preparation using Carbopol.

Carbopol is first hydrated by placing in a beaker of water and stirred at room temperature for 24 hours. Once hydrated, the drug loaded micro-sponges and glycerin are added to the mixture and stirred at 25 RPM. The pH of the gel is adjusted using triethanolamine and entrapped air is removed by allowing to sit overnight. The gels pH would not be a concern, as the pH of semen is very similar to the product we are formulating.(40, 41)

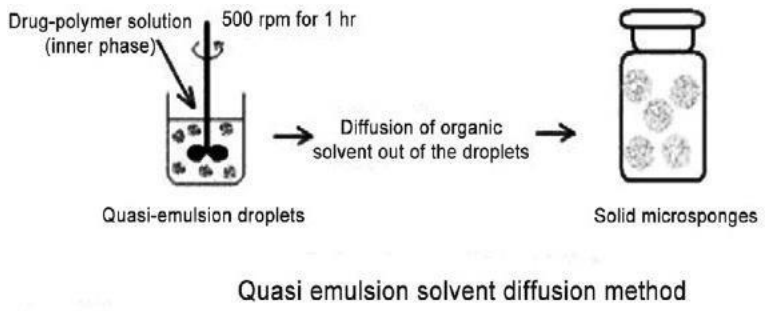

Figure 2: Quasi emulsion solvent diffusion method (46)"

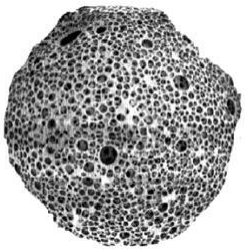

Figure 3: Microsponge structure(47)

2.3.6 VVA rat animal model.

To induce a hypoestrogenic state and thus VVA, it is appropriate to use ovariectomized rats. Briefly**,** Adult nonpregnant female Sprague-Dawley rats 8-10 weeks old were housed 7 days before the surgical procedure. After 1 week of acclimation period, the rats were bilaterally ovariectomized under 1% isoflurane. Post-surgery, it approximately takes 3 weeks to induce an artificial menopausal state, which can be used as an excellent VVA model.(42, 43) In the following study a vaginal gel with ZP-025 was used to treat vaginal atrophy. Briefly, rats were divided into four groups and received 50 μL of the following intravaginal treatments, delivered via a micropipette twice a day (8–10 hours intervals) for 4 weeks: in the control group, rats did not receive any treatment but a micropipette tip was introduced into the vagina; in the placebo group, rats received ZP-025 gel without colostrum; in the treated groups, rats received ZP-025 gel

containing 0.5% or 2.3% of bovine colostrum. Treatment with the ZP-025 gel improved vaginal blood flow and vaginal tissue thickness in the ovariectomized rat model resembling vaginal atrophy. Based on the results of this study, this model can be used to represent VVA in an in-vivo animal model.

3.0 Approach

3.1 Aim 1 will develop and characterize a novel Ospemifene micro-sponge-based gel.

3.1.1 Development of Ospemifene micro-sponges Rationale: To establish an efficient and reliable micro-sponge drug delivery system.

Experimental Design: Initially, Eudragit RS 100 will be dissolved in 2.5 ml of ethanol (soluble in ethanol) and Ospemifene will be added (drug to polymer ratio 4:1) and dissolved under ultrasonication at 35°C for 10 minutes. Outer layer consisting of a mixture of PVA, and water is prepared by adding Eudragit/ospemifene to PVA/water The mixture will be stirred at 500 rpm for 2 hours until it is visually homogenous. (45) Following this, it will be filtered to separate the formed micro-sponges. Finally, the micro-sponges will be dried in an air- heated oven at 40°C for 12 hours. To ensure batch to batch consistency for the micro-sponge preparation method, the experiment will be carried out in triplicate. Additionally, to understand the release profile of the micro-sponges we will perform a dissolution study using basket apparatus I. A known amount of micro-sponges will be placed in the basket and submerged into 150 ml of vaginal stimulant fluid (VFS) rotated at 15 RPM with a temperature of 37 °C.

Analysis: The Quasi-Emulsion method can be deemed successful upon testing/examination of three crucial parameters: entrapment efficiency, drug content and production yield. Production yield (%) is calculated by dividing the weight of micro-sponges obtained by the initial dry weight of the starting material X 100. Entrapment efficiency (%) is calculated by dividing the actual amount of Ospemifene (obtained from drug content results) in the

micro-sponges by the total amount of drug added during the preparation of the micro-sponges X 100. Drug content (%) is calculated by dividing the actual amount of Ospemifene in the micro-sponges by the weight of the micro-sponge powder X 100. To obtain the actual amount in the micro-sponges, we weigh a certain amount of micro-sponge powder and dissolve it in methanol (soluble in methanol)(45). Next, we filter it, and analyze its content using the HPLC method mentioned in our preliminary data section. All parameters will be expressed in percentages and values of the parameters will be compared from batch to batch using students t-test, with a p- value <0.05 considered significant. Samples from the dissolution study will be taken over a period of 5 days (0,1,2,4,6,12,24 hours then daily up to 5 days). Aliquots taken will be replaced with the same amount of media to maintain sink conditions. These samples will be analyzed using the HPLC method mentioned in the preliminary data section. A plot of % drug release vs time will be constructed and used to determine whether our formulation is a following a controlled/sustained release profile.

Expected Outcomes: Influencing factors for production yield, EE and DC are the drug: polymer ratio, volume of organic solvent, and the amount of PVA. According to existing data, we choose a 4:1 drug: polymer ratio, 2.5 ml of ethanol, and 25 mg of PVA dissolved in water because they gave the best results when preparing miconazole micro-sponges, a drug with very similar properties to Ospemifene. We expect that this method of micro-sponge preparation will give us a high production yield (>85%), entrapment efficiency (>85%), and drug content (>85%). Additionally, from our % drug release vs time plot we expect the micro-sponges to slowly release Ospemifene due to its limited solubility in VFS and large particle size. We predict a zero-order release that is maintained up to 5 days. These micro-sponges will be further evaluated for their successful incorporation into the final vaginal gel.

Significance: It's important to manufacture micro-sponges that are able to efficiently trap the drugs within them, therefore studies that show that our method was successful in trapping Ospemifene is important. Additionally, we propose a study to test the release profile of Ospemifene from the micro-sponges in a vagino-mimetic media which will aid us in establishing the rationale behind using of micro-sponges, which in part is due to their ability to establish a controlled/sustained release system.

Pitfalls & Alternative Strategies: Ethanol was chosen as an organic solvent because it has been established that it can readily dissolve Eudragit RS 100. However, its ability to dissolve a high amount of Ospemifene has not been ascertained. Alternatively, dichloromethane (DCM) has been used as an organic solvent to dissolve many drugs in the inner phase of the micro-sponges. It is less polar than ethanol, which may make it better solvent for dissolving Ospemifene. Additionally, DCM is more volatile, which helps with establishing a more rigid micro- sponge complex compared to ethanol. This aids with controlling and sustaining drug release. Another problem that may be present is the low production yield, entrapment efficiency, and drug content. To address this issue, it is important to recognize that a higher drug to polymer ratio usually improves these parameters, and this could be used as an initial solution. Alternatively, its known that PVA is an emulsifier that can dissolve hydrophobic drugs and therefore wash them away, which could decrease all the previously mentioned parameters. Decreasing the amount of PVA could also be an option. Finally, the organic solvent volume affects drug placement and diffusion in the matrix, reducing the volume will also improve the value of the parameters. If drug release is rapid, this problem can be controlled by measuring the particle size and porosity of the micro-sponges. Increasing the particle size by modifying the drug: excipient ratio and organic solvent used will further retard drug release. Additionally, the number and size of pores would need to be

decreased in order to sustain drug release. Porosity is influenced by the amount of PVA, volume and type of organic solvent.

3.1.2 Formulating Ospemifene vaginal gel

Rationale: In order to deliver Ospemifene vaginally for the treatment of VVA symptoms, it needs to be delivered as a gel, which is going to be developed in this section of the proposal.

Experimental Design: The gel is going to be made using Carbopol ETD 2020 as a gelling agent. Briefly, Carbopol will be added to a mixture of water and glycerin to get a concentration of 0.8% of Carbopol. After yielding a homogenous mixture, the micro-sponges will be dispersed in the Carbopol base to get 60 mg of Ospemifene/ 1 g of gel, hyaluronic acid (200mg/1g of gel), and micronized progesterone (100 mg/1g of gel). After the gel is made, triethanolamine is added to neutralize and obtain a pH of 7.0. Once the gel is made, it will be evaluated for color, consistency, appearance, pH, viscosity, bioadhesion, drug content and release. pH will be determined using a digital pH meter. Viscosity and rheological properties will be evaluated using a rotational rheometer at 25°C and 37°C. Bioadhesion is measured by placing a hot-agar mucin solution on a glass plate and letting it solidify at 4-8°C. Once it solidifies, the solution is heated to 60°C to dissolve the agar, which converts it to a mucin-alone solution; this is allowed to equilibrate at 25°C and normal humidity levels 1g of the gel is then placed on the glass plate and held vertically for 20 minutes.The bioadhesion study will also be carried out for a Miconazole marketed gel group (this product was chosen because it has a similar target viscosity profile). For drug content, 250 mg of the gel is taken and dissolved in methanol, filtered, and placed into HPLC vials. Moreover, a replicate of the dissolved sample will be taken and analyzed for progesterone drug content. In-vitro drug release will be done using a two compartment Franz cell system using a dialysis

membrane with a diffusion area of 3 cm^2. 100 ml of VFS is placed in the receptor compartment, which is stirred using a magnetic stirrer and maintained at 37 C. Three different test groups were designed for this study (Ospemifene micro-sponge gel, Ospemifene alone solution, Ospemifene micro-sponge solution). Aliquots will be taken over a period of 24 hours (0 min, every 30 min for 6 hours, followed by 12, 18, 12, 18, 24 hours) and compensated with the same volume of VFS.

Analysis: Color, consistency and appearance will be examined visually. pH and viscosity are reported directly from the instrument. Bioadhesion is measured based on the distance in cm that the gel travelled across the glass plate due to gravity. Drug content and drug release samples for Ospemifene and Progesterone will be analyzed using the HPLC method mentioned in the preliminary data. Drug content will be expressed as the amount obtained/theoretical amount in the gel. Additionally, drug release data for both Ospemifene and Progesterone analyzed via HPLC (cumulative amount released vs time) will be plotted and fitted into a kinetic model (Zero order, first order, Higuishi, and Kormeyer–Peppas) the R^2 of the plot of K values is used to determine which model fits best.

Expected Outcomes: We expect that the gel will be viscous (600-800 P) which is lower than the viscosity the marketed Miconazole gel (around 1000 P). Additionally, the gel is expected to be consistent, clear and transparent. The pH should be 7.0 which is ideal for having a viscous Carbopol gel. Given its thick, viscous nature and the type of Carbopol used, we expect that the gel adheres well to the mucin and a longer time compared to the metronidazole gel product in the study. This will help prolong its action inside the vagina. We expect the drug content to be within acceptable ranges (at least 85% of the theoretical amount). Regarding the drug release, we expect to see a retarded drug release with the Ospemifene micro-sponge gel, which is more pronounced compared to the micro-sponge dispersion and drug only suspension. We expect the gel to follow

a Higuishi kinetic model, whose release is controlled by the erosion of the polymer chains.

Significance: To make a gel that can sustain release for at least 24 hours, it is important to use polymers that can extend the release. Polymers such as Carbopol work synergistically with the micro-sponges. In addition, the glycerin and hyaluronic acid used will relieve the symptoms of VVA acutely until Ospemifene fully works. These experiments are important in order to ensure the use of gels for further studies, that includes tests for its key function: sustained drug release.

Pitfalls & Alternative Strategies: The gel might not have the target pH; this can be solved by adjusting the volume of triethanolamine used. Furthermore, the viscosity can be adjusted by changing the amount of Carbopol & glycerin used or by lowering the pH of the gel. Bio adhesion is dictated by the viscosity of the gel, therefore if the gel did adhere to the mucin, we would have to adjust its viscosity. The drug content might not be within acceptable ranges (85-115% of actual amount). In this case, we must make sure that the extraction procedure and HPLC method are suitable and that there are no factors influencing the drug content. The drug release might not be sustained for a 24-hour period. In this scenario, we have to reduce the initial dumping that could possibly happen, by further adjusting the micro-sponges (particle size and porosity) or changing the amount of excipients used to make the gel. Additionally, certain factors that may influence the viscosity such as the presence of fluids in the vagina, which may dilute the gel and alter its viscosity and bioadhesion. Therefore, it is important to carry out the same studies under conditions where the gel is diluted in different volumes of cervicovaginal fluids, semen, and menses. The presence of a fair amount of shear in the vagina, especially during sexual intercourse may also impact the viscosity and bioadhesion of the gel, therefore a study that assesses the effect of the shearing forces on the gel would be necessary to exclude the influence of shear on the properties of the gel.

3.1.3 Drug product stability evaluation under vaginal conditions

Rationale: Drug products exposed to the vaginal environment are prone to physical and chemical degradation by many different factors, which include temperature, pH, hydrogen peroxide, and enzymes.

Experimental Design*:* Ospemifene micro-sponge gel of will be exposed to harsh conditions which include hydrogen peroxide 3%, 1 N NaOH solution,1 N HCL solution, and to three enzymes commonly present in human cervicovaginal secretion environment: aminopeptidase *100 U*/*mL*, lysozyme 100,000 *U*/*mL*, and proteinase K 100 U/ml. Briefly, 1g of the gel will be incubated in every condition described above at 37°C and sampled over a period of 3 days (0,6,12,24,48,72 hours). In addition, to supplement our experiment, we will acquire human vaginal fluid (HVF) from multiple female donors. Controls will be composed of drug samples that are not exposed to any harsh condition.

Analysis: All our analysis will be done using HPLC. We will look for degradation peaks on the chromatograms and changes in absorbance compared to the control. Ospemifene concentration over time will be reported as Ospemifene recovery (% of time zero) ± standard deviation for all the analyses involving Ospemifene stability assayed with HPLC. The data that will be generated will be compared using one-way analysis of variance (ANOVA). P-values of < 0.05 will be considered statistically significant.

Expected Outcomes: All samples treated in harsh conditions will be compared to controls, in terms of chromatogram degradation peaks and drug percentage recovery. We expect that Ospemifene will have no significant difference from the control. Concentrations of 1 N HCL, 1 N NaOH, 3% Hydrogen peroxide used are mild and closely resemble vaginal conditions. Additionally, the enzymes present in the vagina cleave proteins and peptide bonds, which are not

present in our gel. Therefore, these conditions should not significantly alter Ospemifene's stability.

Significance: Small molecules are prone to physical or chemical degradation and identifying the mechanism by which this degradation may occur will aid us in developing an effective protection strategy.

Pitfalls & Alternative Strategies: Studying in vitro stability will provide the confidence required to formulate a drug product. However, certain conditions were not considered (presence of certain structural diseases and menses) and they may affect drug stability. Therefore, it is important to carry out this experiment under dry, wet (different volumes) and presence of menses to observe any stability changes under these conditions. Additionally, this study does not include account for the role of CYP enzymes, which are known to be present in the vaginal compartment. Studies that incubate CYP enzymes with both Ospemifene and Progesterone are warranted to understand the effect of CYP metabolism on both drugs.

3.1.4 : Drug-Excipient compatibility for the gel

Rationale: To understand whether there are any incompatibilities between the drug and excipients in the gel. **Experimental Design*:*** Differential scanning colorimetry (DSC) and Fourier transformer infrared spectroscopy (FTIR) will be used to evaluate drug-excipient compatibility. DSC will be done for pure drug, each excipient separately, drug-micro-sponge, and gel formulation. Samples will be weighed and placed in aluminum pans and exposed to a heating rate of 10 C/min at a temperature range of 25-450°C in the presence of nitrogen. FTIR will be carried out in a Potassium Bromide disc.

Analysis: For DSC, we will be looking at the thermogram (Heat flow vs Temperature) of each group and comparing the peaks present. For FTIR, the IR-spectra of the 3 groups will be

compared in terms of peaks.

Expected Outcomes: We expect that the presence of a micro-sponge matrix will omit the exothermic peak (if the drug is crystalline). This is due to the homogeneous mixing and integration of the drug within the micro- sponge. We do expect a decomposition and glass transition peak on the thermogram. For FTIR, we expect that the drug alone will have high band peaks due to the presence of various functional groups. Both the formulation and micro-sponge alone will have less observed bands due to the restriction inside the formulation and micro- sponge. Overall, no new band peak should appear or disappear in both micro-sponge only and gel formulation groups.

Significance: It's important to make sure that Ospemifene does not interact with any of the excipients present in the gel which may be a cause issues with stability, efficacy, and safety.

Pitfalls & Alternative Strategies: The excipients used have been reported to not interact with many other active pharmaceuticals in different formulations. If the results hint at a about a possible drug-excipient interaction, we can confirm it by doing additional compatibility studies with Thermogravimetric analysis, X-ray Diffraction, HPLC or LC-MS analysis and looking at peak purities. In case of any incompatibility, the excipient should be identified and replaced with an excipient that is less likely to interact. Alternatively, if the presence of incompatibility does not affect the stability of the formulation no further changes or actions needs to be taken.

3.2 Aim 2 will investigate the lead gel formulation for ex-vivo efficacy and safety.

3.2.1 Ex-vivo efficacy and safety of the Ospemifene gel on a reconstituted vaginal epithelium tissue model

Rationale: To determine the effects of the Ospemifene micro-sponge gel of vaginal epithelial tissue.

Experimental Design: Tissue composed of ecto-cervical vaginal epithelial cells grown on a polycarbonate filter (serum-free special media) are obtained and grown according to the previously reported method.(44) Before treatment, these tissues will be placed in a 6 well plate and incubated at 37°C and 5% CO^2 for 24 hours. During treatment, the media will be replaced with either Ospemifene micro-sponge gel, Ospemifene drug solution in media and Nonoxynol-9 (N-9) gel, Estrogen gel, or new fresh media. There will be three different time points (1,3,5 days). Thickness of tissues will be recorded prior to the experiment. Additionally, to understand the effectiveness of HA, Immunohistological staining will be performed according to VitroScreen procedure using primary and secondary antibodies (Anti-CD44 and Alexa Fluor 488 goat antimouse). An additional group of HA in solution will be used as a positive control.

Analysis: The tissues will be washed, and viability will be evaluated using luminescence cell titer glo assay. Additionally, microscopy will be carried out to assess tissue histology and compared with the controls for any changes. All groups will be compared to controls (no treatment group) for viability using the students t-test, and comparison among different treatment groups will be done using one-way ANOVA with a p-value of <0.05 considered significant. Immunofluorescence (IF) from the tissues will be observed using a fluorescence microscope.

Expected Outcomes: We expect that with both Ospemifene drug solution and formulation,

there will be an increase in thickness and viability in the tissue layer compared to the untreated group. Similarly, we expect that the Ospemifene containing groups will have no significant difference in viability compared to the estrogen control group. This is due to the fact that Ospemifene is a SERM, which will induce an agonistic effect on the estrogen receptors present on the reconstituted vaginal tissue similar to estrogen. We expect that Ospemifene drug solution and formulation will not cause any safety concerns based on the viability and gross histological imaging compared to the N-9 control group.CD44 expression according to the IF imaging will be higher in groups containing HA compared to the negative control and the drug solution group only.

Significance: It is important to make sure that Ospemifene can exert direct effects on the vaginal surface while not having induced any detrimental changes on the ex-vivo model. This step is crucial towards moving the product forward to the animal studies.

Pitfalls & Alternative Strategies: The tissues used in this study are healthy, which may not reflect the true nature of atrophic tissues. Use of atrophic tissues may produce different results. Partial atrophic tissues can be produced by altering the growth conditions of the tissues (Increasing temperature and incorporating a higher degree of humidity). The timepoints used in this study may not be sufficient to observe the long-term effects of Ospemifene. Cellular growth is still expected to happen (increase in viability) but the long-term effect of Ospemifene (resolving the symptoms of VVA) won't be studied in this model.

3.2.2 Ospemifene effect on cellular viability in CaSki and VK2 cell line

Rationale: In order to use Ospemifene micro-sponge gel vaginally, we need to determine its safety by evaluating its effect on the viability of two cervical/vaginal cell lines.

Experimental Design: Both CaSki and VK2 cells will be seeded in 96-well plates at a

density of 1×10^4 cells per well, respectively. After 24 h of incubation at 37°C, the growth medium was replaced with 200 µl medium containing the drug samples with concentrations ranging from 0.1 ug/ml to 200 µg/ml. For the formulation, a known weight of the formulation will be diluted in the growth media to a series of 8 levels and 200 ul will be placed in each well plate. Estrogen gel, drug solution, and 10% SDS will serve as positive controls for this experiment, media only as negative controls.

Analysis: After 24 hours, cell survival (viability) will be measured using cell titer glow assay. Briefly, drug- containing medium will be removed and 100 µl of fresh media and 100 µl of the cell titer glow reagent were added to each well. The plates were shaken for 2 minutes and then left out in room temperature (covered from light) for 10 minutes. The luminance of each plate was then recorded using a microplate reader. Using GraphPad, a sigmoidal relationship between log concentration and viability can be drawn to get the CC50 of the drug/formulation.

Expected Outcomes: We expect that the drug would increase the viability of both cell lines, given its similar mechanism of action to estrogen, which has been reported to cause this kind of phenomena. The formulation may cause a drop in viability at high concentrations, due to the presence of large particles hindering the growth of cells. We expect high CC50s with the drug solution and lesser with the formulation.

Significance: It is important to know that the components or the concentration of drug used in the formulation are not harmful to cervico-vaginal cell lines in order to advance the product into animal studies.

Pitfalls & Alternative Strategies: The series of drug concentrations used may not be suitable and may scramble the results (insolubilized vs solubilized drug). It is important to have a drug concentration range where the drug is soluble. In this case, we may use an acceptable

concentration of DMSO (<0.1%) to enhance the solubility of the drug. Additionally, in case of a low cell count, it is important to make sure that the cells are growing, confluent and that 24 hours were sufficient to get an appropriate cell count and that the growth media is usable and not contaminated.

3.2.3 Compatibility between Ospemifene and lactobacillus

Rationale: In addition to tissue viability, lactobacillus compatibility will be investigated to determine the safe vaginal use of the Ospemifene formulation**.**

Experimental Design: Normal vaginal flora predominantly consists of *Lactobacillus* species; four different *Lactobacillus* species will be tested for growth inhibition and toxicity. The species that will be analyzed includes *L. crispatus*, *L. jensenii* , *L. iners*, and *L. vaginalis* in many different strains. To examine lactobacillus compatibility, we plan to do a minimum cidal concentrations (MCC) and Minimal inhibitory concentration (MIC) assay. For MCC, bacterial suspensions will be prepared by selecting isolated colonies and suspending the test organisms in saline to a density of a 0.5 McFarland standard. These suspensions will be further diluted and incubated at 35 C for 30 minutes. Ospemifene drug solution at four different concentrations (0.5 1,5,10 mg/ml) in ACES buffer or Ospemifene micro-sponge gel will be used. They will be plated, allowed to absorb, and then spread over the surface of the agar plate. Plates will be incubated for 24 hours. For MIC, we will be following the agar dilution method provided by the National Committee on Clinical Laboratory Standards. Briefly, a series of different drug and formulation concentrations that are doubled (i.e., 1, 2, 4, 8, 16, 32 $\mu g\ ml^{-1}$, etc.) will be prepared on agar plates. This is followed by placing a suspension of the *Lactobacillus* strain to be tested, which will have a 0.5 McFarland standard on the agar plates. The plates will be incubated (37

C) and examined after 24 and 48 hours. Controls will include the vehicle without the Ospemifene or the formulation, Estrogen gel and Cefazolin as a positive control. All assays were performed in duplicate and repeated on at least 3 separate occasions.

Analysis: To determine whether Ospemifene is killing different *Lactobacillus* species, the MCC needs to be determined. The minimum cidal concentration is the drug concentration required to reduce the viability of a culture by 99.99% (fewer than 10 colony forming units); colonies will be counted manually. MIC is the smallest concentration of drug required to inhibit the growth of bacteria. Bacterial viability will be compared between the drug containing agar plates and the controls. The loss of viability must be < 1 log10 to meet the safety requirement.

Expected Outcomes: *Given its similar mechanism of action to estrogen,* we expect that Ospemifene will be minimally harmful against vaginal flora. We expect that the MCC and MIC of Ospemifene on lactobacillus will be very high and not close to drug concentrations that we will use vaginally. This will be evident by the presence of abundant bacterial colonies on the agar plates, compared to cefazolin which Is a known positive control that inhibits the growth of lactobacillus. If Ospemifene or the formulation: 1) does not result in a loss of viability <1 log10 compared to the control and 2) produces bacterial viability equal or greater than cefazolin we are safe to say that Ospemifene could be safely used vaginally.

Significance: A limiting factor to vaginal use of many drugs is the damage they cause to the beneficial vaginal microflora. Therefore, our results will be critical in establishing Ospemifene as a safe vaginal formulation.

Pitfalls & Alternative Strategies: We are confident about Ospemifene's inability to inhibit the growth of lactobacillus; however certain strains may be susceptible to Ospemifene. To overcome this problem, we will add cefazolin as a positive control, which is known to inhibit the

growth of lactobacillus to a certain extent. Cefazolin will be directly compared to Ospemifene in terms of MCC and visually evaluating at the colonies on the plates. Additionally, if Ospemifene formulation produces similar results to estrogen gel we are certain that the Ospemifene micro-sponge formulation could be used vaginally for further animal studies.

3.3 Aim 3 will evaluate the safety and efficacy of the Ospemifene micro-sponge-based gel on a VVA rat model.

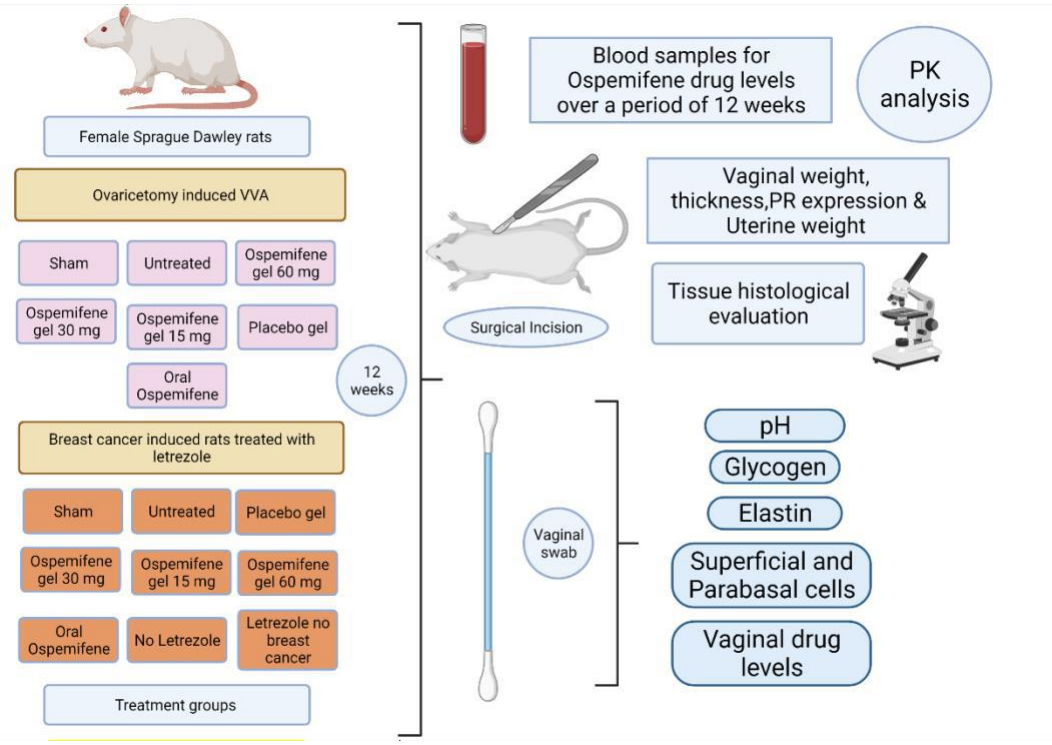

Figure 4: Schematic overview of the different groups and measurements preformed in a VVA rat model.

3.4 Ospemifene micro-sponge gel efficacy and safety in a VVA rat model

Rationale: To test the effectiveness and assess the safety of the novel vaginal gel on an established VVA model.

Experimental Design: Female Sprague Dawley rats were ovariectomized (procedure in preliminary data) and used 2 weeks after the surgery. The experiment compromises of 7 arms (oral Ospemifene 60 mg, vaginal Ospemifene Gel 60 mg, vaginal Gel 30 mg, vaginal Gel 15 mg, placebo gel, untreated and a sham group). Additionally, to understand the efficacy and safety in a combined VVA- breast cancer model, a group of rats will receive 7,12-dimethylbenz(a)anthracene (DMBA) intragastrically by gavage to induce breast cancer and treated with 10 mg of letrozole an aromatase inhibitor. By 8-12 weeks, the rats will receive Ospemifene 60,30,15 mg or placebo gel, Oral Ospemifene, untreated, did not receive letrozole, or receive letrozole with no breast cancer induction.Power analysis (80% power) based on the vaginal weights of the rats to obtain an effect size will be used to determine the number of rats in each tested group. All rats will be randomly allocated to have the mean body weight equivalent among all the groups. Prior to the experiment, each rat was weighted, biopsy was taken from the vagina, and pH was measured. The vaginal formulations will be administered by using a small syringe equivalent to 1g gel (30/15 mg in 1g of gel will be formulated). The oral formulation is dissolved in ethanol, diluted in PEG, and administered using an oral feeding gauge. The placebo consists of the gel without the micro-sponges. The different arms will be administered once daily for 12 weeks. In the 12^{th} week, the rats will have their pH assessed and they will be weighed and sacrificed. Their vaginas (without cervix) and uteruses will be collected. Additionally, part of the vaginal tissue will be stained using H&E to analyze the thickness. The uterus will be excised, weighed, and compared with the control groups. Immunohistology (IH) staining for progestin receptors will be performed by incubating

tissue sections with primary progestin receptor antibodies, followed by horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody. Finally, they will be colored with diaminobenzidine substrate. Moreover, prior to sacrifice, Glycogen, elastin, and drug levels in the vagina will be gathered using a vaginal swab at specific time intervals (0, 6,12 hours 1,3,5 days, 1,2,3,4,6,8,12 weeks). Blood samples (1 ml) will be taken to analyze Ospemifene's systemic exposure (Time points 0,12 hours, 1,3,5 days, 1,3,6,12 weeks).

Analysis: Vaginal and Uterus tissue weight (expressed as rat vagina weight/total body weight) will be compared among all treatment groups and controls. Vaginal thickness and morphology will be analyzed by using microscopy and compared with other groups for changes. Furthermore, from the microscopic imaging, the number of superficial and basal cells will be observed. The IH stained tissues will be also observed under the microscope and semi-qualitatively analyzed based on the number of labeled nuclei: 0, none; 1, low; 2, moderate; 3, high. Glycogen and elastin will be assessed using specific assay kits. Vaginal and Blood concentrations will be analyzed using the LC-MS method provided in the preliminary data section and plotted as concentration vs time (using non-compartmental analysis to obtain AUC, Cmax, Tmax, K and half-life). Descriptive statistics (mean ± standard deviation) will be calculated for all relevant parameters. All group comparisons will be done using one-way ANOVA coupled with Dunnett's post hoc test. Statistical significance was determined at a $p\text{-value} < 0.05$.

Expected Outcomes: In this study, we established two models to access the effectiveness and safety of our gel formulation. We expect that an increase in vaginal weight, thickness, glycogen, elastin and number of superficial cells and a decrease in parabasal cells compared to the controls. Additionally, the groups with breast cancer and VVA will observe a pronounced decrease in symptoms of VVA with any of the treatment groups (orally or vaginally). There would be no

significant difference between oral and vaginal ospemifene formulations. Uterus tissue weight is expected to be the same as the control groups, as the presence of progesterone inhibits endometrial hyperplasia. Progesterone receptors are expected to be upregulated, which is expected by administration of ospemifene, irrespective of the route). The pH increase caused by VVA should be normalized to normal vaginal pH as the condition improves. With the gel, we expect a sustained release in the vagina with minimal drug absorption or presence systematically. When compared to the oral formulation's pharmacokinetic parameters, the gel will display a decrease in both Cmax and AUC and an increase in Tmax. Finally, the surface morphology of the vaginal tissues is expected to be like the sham group (no gross morphological changes). Letrozole is used as a treatment for breast cancer which is known to cause VVA. Ospemifene (oral or vaginal) are able to cause symptomatic relief of the underlying condition by stimulating the estrogen receptors present in the vagina which cause tissue proliferation.

Significance: It is important to test the efficacy and safety of our gel on an established VVA animal model and concurrently to compare the results with the gold standard therapy, oral Ospemifene. This experiment is paramount as it determines the transition of the gel formulation into further clinical development.

Pitfalls & Alternative Strategies: Using a model where breast cancer and VVA are present at same time is representative of the actual disease status. However, the use of an aromatase inhibitor is not always the case in the treatment of breast cancer and other therapeutic options may compete with Ospemifene on the estrogen receptors present on the surface of the vaginal tissues. One example is Tamoxifen, which is used for the treatment of hormone sensitive breast cancer and is known to cause VVA. Therefore, using agents that may impact clinical outcomes instead of aromatase inhibitor is important. With these models, it would be hard to assess the acute effects of the lubricants/moisturizers

used, as there are no subjective measurements for symptoms. In this experiment, we don't know how rapidly the gel is cleared so it may accumulate by the time of the administration of another gel dose. In this case, the administration of another dose is only warranted when there is enough space in the rat's vaginal lumen. Another issue in this experiment is that the concentrations of the active metabolite of Ospemifene is not analyzed, which may be a significant difference between the oral and vaginal formulation. Therefore, it is important to develop an LC-MS method which enables us to analyze the parent drug and the metabolite. In case data was non-parametric we will transform it using a log transformation and analysis it with one-way ANOVA coupled with Dunnett's post hoc test.

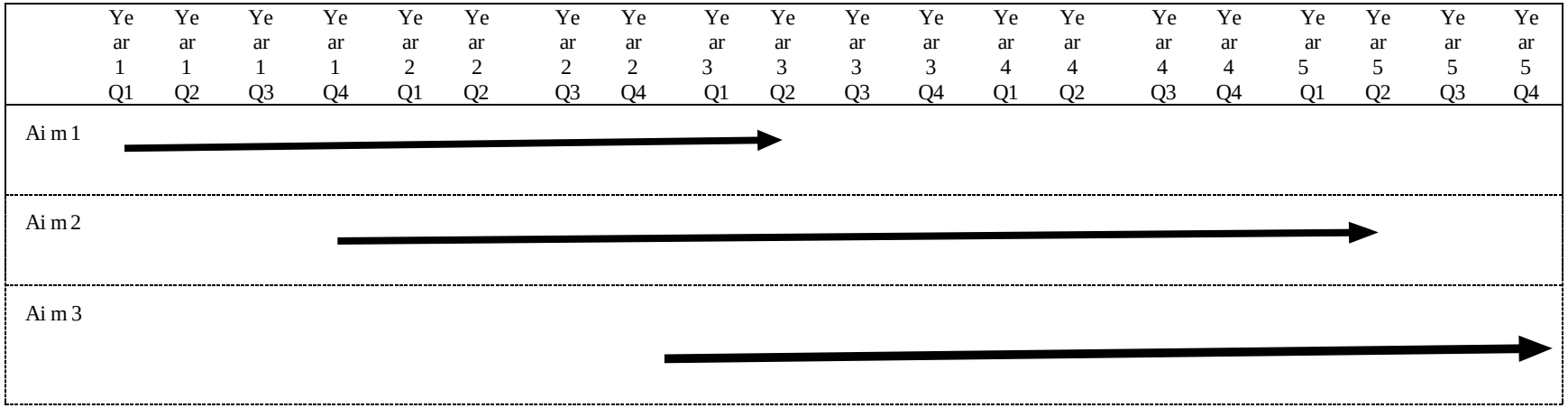

Figure 5: Timeline for the proposal

4.0 Conclusion:

Our ultimate goal is to produce a vaginal gel that is able to treat the symptoms of VVA, convenient for once daily use, and safe with no major or irksome side effects for women with breast cancer. Based on the results of this study, the use of micro-sponges will be promoted, and perhaps could even be incorporated into various therapeutic vaginal dosage forms. These can be used for the treatment of many different vaginal conditions where a safe sustained and controlled formulation is necessary. Additionally, this study will aid in pushing the use of Ospemifene in women with breast cancer where VVA is underreported. The need for drug products that can be safety used for VVA in patients with a history of breast cancer patients is dire. Therefore, the development of Ospemifene as a vaginal product is promising and of utmost need in the upcoming years to address this very bothersome threat to reproductive and public health.

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