Vaginal Microbiota Transplantation: The Next Frontier

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While fecal microbiota transplants (FMT) have received significant attention as a treatment for recurrent *Clostridium difficile*, a new area of microbiota transplantation is emerging: vaginal microbiota transplants (VMT) for treatment of bacterial vaginosis (BV). The recent use of high-throughput genetic sequencing approaches has led to much deeper understanding of the composition of vaginal microbiota communities and their influence on sexual and reproductive health.[1] For example, there has been significant research confirming clinically relevant differences between women with BV, also referred to as polymicrobial vaginal microbiota, and women with dominance by one of only a few lactobacillus species, the latter being considered "optimal." Women with symptomatic BV typically experience abnormal malodor and increased vaginal discharge,[2] although researchers estimate that as many as 50% of women with BV are asymptomatic.[3] There is evidence, however, that even asymptomatic women retain elevated risk of adverse health outcomes.[4] The etiology of BV is not well understood, but a collection of excellent articles recently addressed the current understanding[5] of its pathogenesis,[6] the interplay of its host immunity and environment,[7] and the limitations of its current treatments.[8] There is significant evidence that BV can be sexually transmitted from men to women (i.e., when penile microbiota transferred during vaginal sex disrupt lactobacillus dominance in the vagina) and between female sex partners.[9] There is also evidence that susceptibility to BV is driven in part by the host immune response, which may be altered by numerous environmental, genetic, and hormonal factors.[10]

Researchers have also observed that the prevalence of BV varies by race and ethnicity — demographic data that subjects self-reported. The prevalence of BV has been shown to vary between and within countries worldwide, reported to range from as low as 7%

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in Burkina Faso to as high as 68% in Mozambique.[11] A study of over 4,000 women conducted in the U.S. estimated the prevalence of BV at around 29%, with lower prevalence in non-Hispanic whites (23.2%) and higher prevalence in non-Hispanic blacks (51.4%).[12] Whether this is due to biologic differences originating from genetic variation across racial groups or variation based on ethnicity (e.g. exposure to particular diets, behaviors, and lifestyle factors — whether cultural or socioeconomic) is unclear, given that research methods for collecting demographic data to date often conflate race and ethnicity rather than eliciting more nuanced information.[13] Genetic and biological factors are likely intertwined with differences in socioeconomic status and behavioral factors.[14] Chronic stress, smoking, and certain viral coinfections have all been correlated with increased susceptibility to BV, while hormonal contraceptive use has been correlated with decreased risk of incident, prevalent, and recurrent BV.[15]

Thus, it seems likely that only a bacterial "reset" to a more stable, beneficial lactobacillus-dominated community would have a long-term impact on a woman's sexual and reproductive health. We hypothesize that such a reset could be achieved via vaginal microbiota transplantation (VMT) using cervicovaginal secretions (CVS).

Incredibly, out of the ~180 known species of lactobacilli, only a few (i.e. *L. crispatus, L. iners, L. jensenii, L. gasseri*) have been found to typically dominate the human vagina.[16] However, there is mounting evidence that not all species of lactobacilli found in the human vagina should be considered optimal or beneficial; namely, vaginal microbiota communities dominated by *Lactobacillus iners* have been observed to be less stable and more pro-inflammatory.[17] This is particularly concerning because *L. iners* is the species of lactobacillus most commonly found after "successful" antibiotic treatment for BV, and was most commonly associated with transitions to diverse microbiota.[18] Thus, it seems likely that only a bacterial "reset" to a more stable, beneficial lactobacillus-dominated community would have a long-term impact on a woman's sexual and reproductive health. We hypothesize that such a reset could be achieved via vaginal microbiota transplantation (VMT) using cervicovaginal secretions (CVS).

Medical Considerations

The Clinical Need

The composition of a woman's vaginal microbiota has a profound impact on her sexual and reproductive health and on her susceptibility to disease.[19] BV is a vaginal infection that is tied to the composition of the vaginal microbiota. It is typically identified using Amsel's criteria and Nugent score.[20] BV can be a distressing and chronic condition for many women. For more than 50% of women with the condition, BV negatively affects their quality of life.[21] BV often recurs and is frequently recalcitrant to antibiotic treatment. In addition to physical symptoms, BV can have an emotional impact on women who experience the condition. In one study, women frequently reported that their symptoms "made them feel embarrassed, ashamed, 'dirty' and concerned others [might] detect their malodour and abnormal discharge."[22] Their symptoms also affected their self-esteem and sex lives, making them reluctant to engage in sexual activity. Further, women with BV are more susceptible to sexually transmitted infections (STIs), including human immunodeficiency virus (HIV), gonococcal, chlamydial, and trichomonal infections.[23] STI transmission rates from women to men are also higher if the woman has BV.[24] Further, researchers have found evidence of a continuum of microbiota in the female reproductive tract linking the vaginal microbiota to the uterine microbiota,[25] and thus women with BV are at higher risk for pelvic inflammatory disease, as well as miscarriage, premature delivery, and post-partum endometritis.[26] Similar observations have also been made recently connecting vaginal and bladder microbiota,[27] perhaps explaining increased risk of urinary tract infections associated with BV.[28] Potential links have also been found between vaginal microbiota and cervical, ovarian, and urothelial cancers.[29]

Clearly, BV is a profound concern in women's health, and yet our current approaches for treatment have limited success.[30] The current standard of care is use of antimicrobials with broad-spectrum anaerobic bacterial coverage, such as metronidazole and clindamycin. Short-term cure rates for first line treatments are typically 60-70% at four weeks after treatment, but recurrence rates in excess of 50% occur within the first year.[31] Other drugs based on different mechanisms of action for treatment of BV are in the FDA pipeline, but as of now, none have yet been approved.

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VMT as a Potential Solution

The tremendous success of fecal microbiota transplantation (FMT) for treating *Clostrioides difficile* infection (CDI) has launched interest in microbiota transplantations and microbiota-based therapies for a wide range of conditions and diseases. Whereas FMT involves transfer of fecal matter, VMT would involve transplanting vaginal bacteria from one woman to another using CVS, a mixture of mucus secreted from the endocervix into the vagina, shed epithelial cells, bacteria, and other proteins, ions, and lipids. CVS can be self-collected using a non-absorptive menstrual fluid collection device, a method that is both quick and, unlike other absorptive collection methods such as swabs and cervicovaginal lavage, does not require dilution.[32] Herein, we refer to VMT as the process of obtaining CVS from a female donor, and after some minimal processing with the goal of maintaining viability of the bacteria, administering the donor CVS material into the vagina of a recipient.

To our knowledge, the first reported successful VMT procedures in humans were reported in 1955 by Gardner and Dukes as part of their efforts to identify the bacteria that was thought to cause the condition "nonspecific vaginitis." They reported that they were able to induce *Haemophilus vaginalis* vaginitis (now called BV) in 11 out of 15 volunteers (73%) by directly inoculating material from the vaginas of infected women into the vaginas of the healthy volunteers.[33] Further, in two of the successful inoculations, the donor material had been taken "from patients in whom the disease had been experimentally produced." No details were given as to how the material was collected and transplanted. In contrast, using pure cultured bacteria, the researchers were able to infect only one out of 13 women.[34]

The use of VMT to treat or prevent recurrence of BV would require the transplantation of *Lactobacillus* species from the donor to the recipient. Although we are not aware of attempts to transplant lactobacillus bacteria from a donor to a recipient by VMT, there is significant epidemiological evidence that vaginal microbiota are transferred routinely between women who have sex with women (WSW) through sexual practice. In one study of 58 monogamous female couples, 95% had concordance for the absence or presence of BV.[35] In another study of WSW, women with BV were more likely to report a sexual partner with BV, sharing of vaginally inserted sex toys, and vaginal lubricant use.[36] Another study suggested similar trends with the presence of *Lactobacillus* bacteria; of 31 couples monogamous for more than three months, 23 (77%) were found to possess identical strains of *Lactobacillus*.[37] The likelihood of sharing identical lactobacilli was not related to age, lifetime male sex partners, or the practice, frequency, or timing of other sexual behaviors. The only practice that demonstrated a trend toward association with sharing identical lactobacilli strains was the reported use of shared vaginal sex toys.[38] Another recent study, which was the largest and longest community-based prospective cohort study of WSW, provided additional data to support transmission of bacterial species between women. Coenrolled largely monogamous couples had a low rate of incident BV, and had predominantly lactobacillus-dominated vaginal microbiota that remained closely aligned and stable over long periods of time.[39] These studies support the feasibility of transplanting lactobacillus bacteria from a donor to a recipient using CVS.

Comparison to Other Ongoing Microbiota Transplant Studies Practices

An analogous form of vaginal microbiota transfer, or vaginal seeding, is being tested as a way to expose babies born by Caesarean section (C-section) to their mother's vaginal secretions as would occur during vaginal birth.[40] Several studies implicate birth by C-section in increasing risk of obesity, asthma, allergies, and immune deficiencies.[41] The observation that the composition of the microbiota that colonizes the body of newborns differs between birth by C-section and vaginal birth suggests that this early exposure can play a role in educating the immune system.[42] In these studies, mothers with *Lactobacillus* dominated vaginal microbiota undergoing a scheduled C-section have sterile gauze inserted in their vagina to collect their CVS during the hour prior to surgery.[43] Within the first two minutes of birth, babies are exposed to their mother's CVS by swabbing the mouth, face, and body with the gauze. Early results suggest that the bacterial communities of newborns delivered by C-section could be partially restored to resemble that of vaginally delivered babies.[44] By nature, vaginal seeding is not a true transplant, in that the donor material is not administered to the same site in the body in the recipient.[45]

Although there are clear parallels between VMT and FMT, there are also significant physiological and clinical differences that must be considered when developing treatment protocols, developing a regulatory framework, and measuring efficacy in clinical care and research. In the healthy intestines, the microbiota composition is highly diverse, with roughly 160 bacterial species per person.[46] In the disease state, *C. difficile* is the single causative agent, and more likely to lead to disease in individuals with decreased bacterial diversity, such as those treated with antibiot-

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ics.[47] Thus, the goal with FMT is to inhibit *C. difficile* proliferation in the intestines, which leads to a return to healthy bacterial diversity. In contrast, there is no single causative agent for BV, and it is the depletion of lactobacillus bacteria and resulting overgrowth of a polymicrobial community of anaerobes that is considered pathological.[48] In the case of *Lactobacillus*-dominated vaginal microbiota, usually a single *Lactobacillus* species is highly abundant and desirous for VMT donors. Moreover, when FMT is used for treating CDI, complete microbiota engraftment is not essential for a clinical cure.[49] While the subset of bacteria strains from the donor stool that initially engraft in the recipient wanes over time,[50] at the point where the transplanted strains are at their nadir, the CDI would be eradicated and recurrence less common. In the context of BV, the *Lactobacillus* species that repopulate after antibiotic treatment, commonly *L. iners*, typically do not provide protection from BV recurrence.[51] Thus, the goal of VMT may be engraftment of the dominant *Lactobacillus* species in the donor sample as well as the minor bacterial species in the community. Although the dominant species may be the most obvious important player, the other minor species may play an important role in allowing the recovery of the dominant species after perturbations due to menses and sexual activity.[52]

Regulatory Considerations

Regulation and Oversight

For decades, the U.S. Department of Health and Human Services, through the Food and Drug Administration (FDA), has overseen the approval of drugs, biologics, and medical devices for commercial distribution by requiring demonstration of safety and efficacy through clinical trials research. However, such FDA oversight is contingent on the substance being tested, e.g., it must be a drug, biologic or medical device and it must have a connection to interstate commerce. In the early days of FMT, many physicians did not think that fecal matter was a drug, nor did they anticipate that it would be a product that would be marketed. Therefore, they believed FMT would not be subject to FDA oversight. Instead, they viewed FMT, with stool typically provided from a local donor known to the patient, as the practice of medicine. In fact, the first recorded use of FMT was documented in 1958,[53] but the first randomized clinical trial for treating recurrent CDI with FMT was reported in 2013.[54] In the interim, FMT was conducted by physicians as part of clinical care. In 2013, FDA stated that it considered fecal material to be a "live biotherapeutic product" (LBP), a subcategory of drugs, and that physicians performing FMTs would need to submit an Investigational New Drug application (IND) to the agency. However, in response to a groundswell of opposition, FDA announced that it would exercise its "enforcement discretion" and not require an IND for physicians performing FMT for patients with recurrent CDI unresponsive to standard antibiotic therapy.

Although VMT is newer in the repertoire of microbiota transplantations, many of the same initial regulatory questions apply to VMT as to FMT. An initial issue is whether the FDA has regulatory authority over VMT. As stated above, FDA jurisdiction is tied to products that have a connection to "interstate commerce" and, in addition, are "held for sale."[55] Prior to the 1997 Food and Drug Modernization Act (FDAMA), there might have been a successful argument that FDA does not have the authority to regulate VMT with vaginal secretions from a donor known to the patient and performed in a doctor's office. Rather, the procedure might have been the practice of medicine, regulated by state medical boards. However, FDAMA made clear that "the connection with interstate commerce required for [FDA] jurisdiction" is presumed to exist[56] and case law since the passage of the act has expanded the domain of what counts as interstate commerce. This includes products that have never crossed state lines but include some ingredient or component part that has traveled in interstate commerce.[57] In addition, the product need not be sold nor must there be an intent to sell the product. As long as the product is used by a physician in a procedure it can be considered "held for sale."[58] Thus, in the case of VMT, a court would likely find that although the CVS are not a part of interstate commerce, the collection device would be, as a component part of the VMT, and therefore the agency would be able to assert jurisdiction. While some would consider this regulatory overreaching, the federal courts in a number of circuits have embraced FDA's broader jurisdiction. Only if the procedure were to be performed for free would it possibly be considered outside of FDA's jurisdiction, but even then, courts have considered devices used by physicians in the course of a procedure "held for sale."[59]

If CVS were to be sold, it clearly would be subject to FDA rules and regulations. In the context of FMT, this is being played out in FDA's efforts to regulate stool from a stool bank that is shipped across state lines and is sold to physicians and hospitals for treatment of recurrent *C. difficile* that is unresponsive to traditional antibiotic therapies. It is conceivable that CVS, like stool or blood, could be banked and sold to health care providers and patients. Similar to stool, CVS is self-collected, though the timing for collection is not dependent on unpredictable bodily functions and, thus, can be more flexible. While donors would have

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to avoid days like menstruation and ovulation (if not using hormonal contraceptives, as ovulation affects vaginal secretions), collection could occur at any time on eligible days. The basic eligible donor pool would be limited to women of reproductive age, and the stringency of the screening criteria would be at least as high for CVS collection as has been reported for stool. In contrast to blood collection, self-collection of CVS is minimally invasive, similar to tampon insertion and removal, and does not cause pain or have risks of side effects such as dizziness, fainting, etc.

In addition to having a connection to interstate commerce, FDA jurisdiction to regulate a substance as a drug or biologic is tied to whether it is to be used to cure, treat, mitigate or prevent symptoms of disease. Some researchers and clinicians believe that a vaginal microbiota not dominated by lactobacillus should be considered "diseased."[60] Under this definition, VMT to increase lactobacillus would be a treatment, and the regulatory path would be that for a drug or biologic. However, sometimes, women with BV are asymptomatic, which means a VMT might be used to "promote vaginal health." There is also the possibility that VMT could be used with adolescent girls as a prophylactic to promote lactobacilli during the time where the vaginal microbiota starts to potentially shift toward lactobacillus dominance.[61] Such claims, i.e., "promotion of vaginal health" and "promotion of lactobacillus," would be considered structure/function claims in the world of dietary supplements, and the product would not be regulated as a drug, but because CVS via VMT is delivered vaginally rather than orally, CVS could not be considered a dietary supplement. The claims would be considered drug claims for prevention of BV. Alternatively, some women might seek out a VMT to reduce vaginal odor. This claim, "eliminating odor," could have different regulatory implications than treatment claims. For example, a product claiming only to reduce odor would be regulated as a cosmetic rather than as a drug.

Under the Federal Food Drug and Cosmetic Act (FDCA), a cosmetic is a product (excluding soap) "intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance."[62] While cosmetics, under the law, may not be "adulterated" or "misbranded," they do not require FDA approval prior to marketing. Products, such as vaginal douches, for example, that claim to cleanse or make a woman "feel fresh," are regulated as cosmetics. However, as stated above, if the purpose of the substance/procedure is to treat, cure, prevent or mitigate the symptoms of a disease or to affect the structure/function of the body, the substance would come within the regulatory umbrella for drugs and biologics. Yet, as has been the case with fecal matter for FMT where a number of researchers and clinicians have questioned the "fit" of the drug/biologic pathway, CVS for VMT poses similar challenges to the drug and biologic regulatory scheme.

Finding the Right Regulatory Pathway

Although FDA has the authority to regulate CVS as a drug/biologic, the drug/biologic approval process assumes that the product can be standardized so that each therapeutic unit is the same in terms of composition, dose and potency. While the microbiota community in CVS may be relatively more homogeneous than stool, it will still differ from woman to woman and will fluctuate over time. Similar to stool, dosing and potency are likely to be difficult to determine. Although some of these challenges are addressed for biologics through the manufacturing process, obstacles still remain.[63]

Another concern with the drug pathway is that because of the expense to the drug manufacturer/sponsor of going through all of the required procedures and clinical trials, the price of the ultimate drug, if approved, is likely to be quite high. In addition, the approval process takes on average ten years. If during this timeframe women want access to the procedure, like the situation with FMT, women may engage in a "do-it-yourself (DIY)" VMT. The potential for "DIY" VMT exists as is evidenced by the fact that women who share sex toys share the same vaginal microbiome as a result of transfer of vaginal microorganisms. Given that the process of using a menstrual cup to collect CVS is as simple as tampon insertion and removal, the methods are publicly available, and the menstrual cups themselves can be obtained over the counter at most pharmacies, the potential for DIY treatment may be higher than with FMT. (Additional challenges to regulating CVS as a drug/biologic through the submission of an IND are described below in the section below entitled "Submitting an IND.")

In the context of FMT, several authors have suggested that regulatory models based on other transferred/transplanted human body constituents, e.g., organs, blood or tissue, might be more appropriate for microbiota transplantations.[64] These other areas are regulated as a combination of the practice of medicine with FDA or the Public Health Service (PHS) oversight of the screening of donors and testing of transplanted material for infectious diseases and the regulation of any banks where such products are collected, packaged and stored for use. If CVS banks were to materialize, the regulatory framework for human cells, tissues and cellular and tissue-based products (HCT/Ps) may be a better fit than that for drugs/bio-

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logics. HCT/Ps are defined as articles "containing or consisting of human cells or tissues that are intended for implantation, transplantation, infusion, or transfer into a human recipient."[65] The regulatory framework for HCT/Ps is focused on prevention of communicable disease transmission and safe processing and handling. Thus, it includes detailed rules regarding donor screening and methods, facilities, and controls for manufacturing to prevent contamination and cross-contamination.

FDA classifies HCT/Ps into two groups: Section 361 Products and Section 351 Products. (Sections refer to the PHS Act, which addresses prevention of the introduction, transmission, or spread of communicable disease.) Section 361 products are considered less risky than Section 351 products and are less tightly regulated. To be considered a 361 HCT/P, the product must be "minimally manipulated" and must be intended for homologous use (i.e., perform the same use or basic function as in the donor) as determined by labeling and advertising or other indications of the manufacturer's intent. The definition of "minimal manipulation" depends upon whether the HCT/P is a structural tissue, as opposed to cells or nonstructural tissue. For nonstructural tissue, FDA defines "minimal manipulation" as "processing that does not alter the relevant biological characteristics of cells or tissues."[66] In addition, its manufacture must not involve combination with another article, except for water, crystalloids, or a sterilizing, preserving, or storage agent. Nor can it have a systemic effect or be dependent upon the metabolic activity of living cells for its primary function, or, if it has a systemic effect or is dependent on "the metabolic activity of living cells for its primary function," it must be intended for autologous use or allogenic use in close relatives (first or second degree blood relatives) or for reproductive use.[67]

CVS delivered by VMT appears to meet each of the criteria for a Section 361 product. However, as regards the last criterion, because of the potential systemic effect on the recipient and because the primary action of CVS would be dependent on the metabolic activity of living cells, it would have to be limited to use in close relatives. This latter criterion would be an obstacle to banking CVS, but FDA could amend the requirement to allow for broader use with adequate regulation of CVS banks.

The regulatory scheme for Section 361 products includes: 1) registration of facilities and submission of a list of all products to FDA; 2) donor screening and testing; 3) current good tissue practices; 4) labeling; 5) adverse-event reporting; and 6) inspection and enforcement.[68] Establishments that manufacture an HCT/P must register and submit a list of every HCT/P that is manufactured in the establishment. This provides FDA with a list of facilities that it may then inspect to ensure compliance with all regulations. All cell or tissue donors must be screened for risk factors of relevant communicable diseases. In addition to donor screening, the specimen to be donated must also be tested for specific diseases.[69]

Good tissue practice refers to the recovery, processing, storage, labeling, packaging and distribution of the product. The focus is on ensuring not only that the cells or tissues do not contain communicable disease agents but also that they are not contaminated in the manufacturing process.[70] Manufacturers must also track each HCT/P so that in case of an adverse event, the root cause may be investigated.[71] An HCT/P that meets the criteria for regulation *solely* under section 361 of the PHS Act is not subject to premarket clear-

At this time, while FDA has not issued regulatory guidance on CVS/VMT for treatment of BV, it is likely that the agency would, at least initially, consider it a drug/biologic and require an IND application for human subjects research. In our experience, the IRB at Johns Hopkins viewed the issue similarly, and required communication with the FDA prior to considering study applications. Thus, we (LME, KD, FZ, and collaborators) have worked with the FDA to establish a framework for screening potential CVS donors, as well as procedures for handling, storing, and performing quality control checks on CVS. Based on our experience, we describe here some of the issues that may arise for researchers in fulfilling the IND requirements.

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ance or approval. HCT/Ps that do not meet the criteria for regulation *solely* as a 361 HCT/P are subject to an additional layer of regulation under section 351 of the PHS and under Sec. 505B of the federal FDCA governing biologics.[72]

Submitting an IND

At this time, while FDA has not issued regulatory guidance on CVS/VMT for treatment of BV, it is likely that the agency would, at least initially, consider it a drug/biologic and require an IND application for human subjects research. In our experience, the IRB at Johns Hopkins viewed the issue similarly, and required communication with the FDA prior to considering study applications. Thus, we (LME, KD, FZ, and collaborators) have worked with the FDA to establish a framework for screening potential CVS donors,[73] as well as procedures for handling, storing, and performing quality control checks on CVS. Based on our experience, we describe here some of the issues that may arise for researchers in fulfilling the IND requirements.

In addition to the clinical trial design aspects discussed below, an IND application must include information about the Chemistry, Manufacturing, and Controls (CMC) of the drug or biologic. For CVS, this could include key physicochemical properties, such as pH, lactic acid content, and the amount of lactobacilli bacteria present (colony forming units, or CFU) before and after freezing. Additionally, an IND generally includes a summary of the pharmacological, toxicological, and biological disposition of a drug in animals in addition to the extent known in humans. However, the use of preclinical animal models for studying vaginal microbiota is severely limited by the fact that the human vagina is uniquely dominated and acidified by lactobacillus species, and the role of lactic acid in maintaining human vaginal health is well-established.[74] Researchers have demonstrated that it is possible to temporarily colonize the mouse vagina with exogenous lactobacilli, but it requires sustained estradiol treatment, the colonization only lasts a few days, and the bacterial concentrations are as much as 1,000 times lower than in human CVS. Thus, the bacteria do not effectively acidify the vagina.[75] Similarly, our primate cousins do not have vaginas dominated and acidified by lactobacilli.[76] It has been suggested that the normal rhesus macaque genital microbiota shows similarities to that of humans with BV.[77] Thus, it is not feasible in animal models to study vaginal microbial dynamics, VMT, or factors that facilitate beneficial bacterial colonization in the human vagina. This is just one example of how typical IND requirements do not apply to VMT with CVS.

Another key element of an IND is the clinical protocol. Our team at Johns Hopkins (LME, KD, FZ, and collaborators) has designed a clinical study to determine whether vaginal microbiota can be engrafted from one woman to another in a controlled clinical setting, and whether the process is safe and well-tolerated.[78] We discuss a few of the factors we considered in designing the protocol below.

Selection of VMT Donors

A key difference between FMT and VMT is the disease target for treatment. If we consider the primary use for FMT, treatment of recurrent CDI, the patients receiving treatment are generally older (two out of every three healthcare-associated CDIs occur in patients 65 and over[79]) and can die from complications of the disease. In contrast, BV is a non-lethal condition that affects reproductive age women, so the risk-to-benefit ratio is less tolerant of risk to the recipient. Thus, careful screening of the donor participants for VMT is of paramount importance to avoid exposure to infectious agents. Our donor screening approach[80] combines the FDA guidance for screening donors for HCT/Ps[81] with testing for additional STIs, fungi, the TORCH infections (toxoplasmosis, rubella, cytomegalovirus, etc.) associated with congenital anomalies,[82] and general measures of immunocompetence. Screening questionnaires include the standard set of questions asked prior to blood donations, sexual and reproductive history, and behaviors that have been correlated with alterations in vaginal microbiota. Travel exclusions include travel by the participant or a sexual partner to regions or countries where Ebola or Zika outbreaks occurred in the past 12 months.

Similar to the model developed by the stool bank OpenBiome,[83] donors that pass the initial screening will provide multiple CVS samples that will be frozen until confirmatory testing. Each CVS sample from the donor will further be screened for various swab-based pathogen tests and characterized to ensure acidic pH and dominance by one of the common lactobacillus species. Each CVS sample will be tested for the presence of sperm, and if found, that sample will be excluded. Although the CVS collection procedure poses no inherent risk to women who are pregnant or breastfeeding, as a result of the demonstrated alterations in vaginal microbiota that occur in response to hormone changes during pregnancy, we have precluded those women as donors. Concomitant use of antibiotics for any reason, as well as use of other medications that may affect the vaginal microbiota, may be grounds for exclusion at the discretion of the study physician.

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Selection of VMT Recipients

Similar to testing FMT in individuals with recurrent CDI that is unresponsive to standard antibiotics, we propose testing VMT initially in women who have recurrent symptomatic BV. Although there are many theories as to why BV recurs that make effective treatment very challenging, women with recurrent BV are in the most need of alternative therapeutic approaches.[84] Currently, there is no universally accepted or clinical definition of recurrent BV.[85] In the case of FMT, the clinical recommendations are to use FMT for CDI after a third recurrence.[86] For our first pending clinical investigation, we have defined recurrent BV as having had at least three prior lifetime diagnoses and having received treatment at least once in the past five years. However, it is worth considering that such a narrow definition may be limiting in reaching everyone that may benefit from VMT. Women may not always be aware of their BV, as the presence of symptoms can be highly subjective. Of the four Amsel's criteria for clinical diagnosis, only two (discharge and odor) are observable by an individual, and a woman may consider this to be "normal." In contrast, the other two criteria of pH >4.5 and the presence of "clue" cells can typically only be confirmed in the clinic or laboratory. Thus, not all women with clinically diagnosable BV seek treatment. Some women may cease seeking treatment that does not work long term. Such factors should be considered for future development and clinical application of VMT.

Recipients should also be pre-menopausal, as the hormonal changes associated with menopause cause a shift away from *Lactobacillus* dominance in the vagina that is unlikely to be altered without concomitant hormone replacement therapy.[87] Further, vaginal microbiota and pathogens are known to have an impact on pregnancy and postnatal outcomes. Thus, additional key inclusion criteria for recipients are use of hormonal and/or barrier contraception for heterosexual intercourse during the study, and not currently pregnant, breastfeeding, or planning to become pregnant. Recipient participants should receive condoms and counseling for use and should be screened for the same range of infections as donors, ideally on two separate occasions prior to VMT and at the time of VMT, to define the recipient's pre-VMT infection status. Certain infections, such as HIV and any other condition that may compromise the immune system, yeast, chlamydia, gonorrhea, etc., should be exclusions for VMT. In contrast, certain infections like herpes simplex virus (HSV) and HPV need not be exclusions, but should be clearly defined prior to VMT to document baseline status. The intermittent nature of viral shedding[88] motivates repeated testing of the recipient prior to VMT.

Test Groups

Importantly, for enrollment into a clinical trial, the recipients should be diagnosed as currently having symptomatic BV, and be given standard of care antibiotics, such as vaginal metronidazole gel daily for five days. This can reduce the burden of BV-associated bacteria prior to VMT, and is analogous to antibiotic use with FMT.[89] VMT should occur after an antibiotic "washout period" of a minimum of 24 hours. The placebo group should receive standard of care followed by a placebo treatment of a benign vehicle, such as normal saline. Recipients should also track sexual activity, product use (douches, lubricants, etc.), and other behaviors that may affect the success of bacterial engraftment, as it has been demonstrated that vaginal intercourse decreases likelihood of engraftment[90] and sexual activity is associated with microbiota community fluctuations.[91]

Donor-Recipient "Matching"

With the multitude of factors that could affect treatment success, it may be more informative to test the engraftment of a donor species in multiple recipients rather than a single recipient. Further, the stringency of the screening criteria and the associated costs of testing favor the approach of using fewer donors. However, using one universal donor would be potentially limiting in determining whether a particular *Lactobacillus* sp. is more successful. Thus, we suggest a small group of donors that could provide multiple samples to a larger group of recipients. The concept of "universal donors" also has precedent in FMT.[92] Further, to reduce the compounded risk of infection and other adverse events to the recipient from receiving multiple doses of CVS, we envision that the first study include a single CVS dose at a standardized volume and dilution. Of course, as clinical information becomes available and safety data established for VMT, these clinical study design parameters should be adjusted accordingly.

Endpoints

The FDA guidance for developing BV treatments suggests assessing clinical cure at seven to 14 days and follow-up at 21-30 days.[93] However, as described previously, the "cure" rate at four weeks for antibiotics is typically quite high, while recurrence occurs at a high frequency in the months thereafter. Thus, the need for innovation is in developing therapies that lead to long-lasting resolution of symptoms, which is a goal of "resetting" the microbiota with VMT. Thus, it is important to follow the dynamics of the vaginal microbiota for at least six months following treatment, though obtaining clinical cure within the first four weeks may

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still be an objective. However, for the first exploratory studies where antibiotics are used prior to VMT, the more pertinent primary objective may be to determine whether engraftment of the donor *Lactobacillus* occurs in the recipient. In this case, vaginal probiotic study designs may be informative. Secondary objectives may include assessment of local adverse events, characterization of vaginal microbiota dynamics in each group, and the frequency of BV in each group at one to six months after treatment.

Ethical Considerations

At one level, a research ethics review for VMT clinical trials would involve similar issues as is seen in other studies, such as ensuring adequate informed consent for STI screening and availability of counseling if a donor or recipient tests positive for HIV or other STIs. However, there are unique issues to consider. The fact that BV prevalence varies by race/ethnicity and region raises questions regarding what is captured through "race" and "ethnicity" categorizations. The FDA recommends that ethnicity and race be self-reported by (1) Hispanic/Latino or not Hispanic/Latino and (2) One or more of the following: American Indian or Alaska Native, Asian, black or African American, Native Hawaiian or other Pacific Islander, and white.[94] However, increasing evidence shows that social determinants of health, race, and ethnicity are intertwined. While race is often assumed to be rooted in biology (e.g. genetics) and ethnicity in cultural ancestry, the reality of institutional racism results in health disparities that disadvantage people of color and ethnic minorities. Heintzman and Marino[95] point out that zip code may better predict health outcomes than one's genetic code, and that our ability to understand how race and ethnicity impact biology and health outcomes would be better achieved through linkages of race/ethnicity data to large data sets, rather than analyzing self-reported race/ethnicity alone. Thus, researchers should be thoughtful about the demographic data they collect in order to address issues of race/ethnicity responsibly.

Researchers should also be sensitive to unintended messaging when making study design decisions or analyzing data according to race/ethnicity of donors or recipients. The issue of racial disparity in kidney transplantation may shed light on this issue. African Americans experience end-stage renal disease (ESRD) at a higher rate than others but until recently were less likely than whites to receive a kidney transplant.[96] The higher ESRD rate was caused in part by health care access disparities and other systemic racial biases that adversely affect African Americans. As a result, African Americans have exhibited less trust in health care providers and researchers, and have been less likely to designate themselves as organ donors. This exacerbated the kidney transplant disparity since there were fewer kidneys with a compatible tissue match available to African Americans on the kidney transplant wait list. Changes in how tissue matching now occurs and outreach efforts to boost African American organ donation and allocation algorithms have closed this particular disparity gap.[97] But, to conclude that African Americans were less likely to receive a kidney transplant because too few signed up as organ donors ignores the history of systemic racism that fed this vicious cycle.

Thus, we recommend addressing issues of racial disparity transparently and mindfully when enrolling VMT donors and recipients. When race/ethnicity may be a factor in enrollment, screening, analysis or dissemination of findings, researchers should explain to donors and recipients that the vaginal microbiome differs by race, ethnicity, and region, that there are some known and other unknown reasons for this, and that VMT research aims to explore such questions. The potential for stigmatizing women of a particular race should be carefully thought through, as Havasupai diabetes researchers learned.[98] For example, if findings support higher BV prevalence in black women, the reasons for this should be reported in full context (e.g., that this could be related to higher baseline stress levels among black women and other factors grounded in social determinants of health).[99] Another example might be if a higher BV prevalence was associated with certain sexual practices or poor health outcomes such as low birthweight in their offspring, such findings could contribute to group stigma and thus to group harm.

In addition to the issues of racial/ethnic disparity and group stigma, microbiome research raises similar ethical questions to those posed by genetic research, e.g., predisposition to disease, privacy, confidentiality, informational risks, incidental findings, and individual stigma.[100] For example, disclosing findings from screening procedures may reveal previously unknown information, such as pregnancy or HIV infection. Risks also include inadvertent disclosure of this information to a subject's partner or other family member, or access to the information by unauthorized personnel. Some of these concerns led to the passage by Congress of the Genetic Information Nondiscrimination Act (GINA) in order to protect individuals from possible discrimination by employers and insurers. Depending on the microbiome sequencing approach, human genetic information may or may not be obtained from microbiome samples/donations. Moreover, GINA may not be protective of some of the more

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insidious types of risks associated with microbiome based research, such as revealing information about personal and private sexual practices. Whether GINA protects individuals based on discrimination from disclosure of microbiome-based information has yet to be determined. Stigma of the type described above, i.e., associated with predisposition to BV and related adverse health and reproductive health outcomes, may not be a basis for discrimination by employers or insurers, but may affect an individual's inclusion in social groups or intimate relationships. There is much less our laws or regulations can do about this type of discrimination. Thus, it behooves microbiome researchers to spend time translating their findings to the public in full context to minimize the risk of stigma-related harms.[101]

Lastly, funding and regulatory agencies should acknowledge that the burden of BV and urgency of developing safe and effective treatments should be defined by those affected, namely women who have BV. For example, regulators and those funding research may perceive that less severe BV symptoms (e.g., malodour and itching) do not warrant the same priority and urgency in VMT research as other symptoms and may de-prioritize studies assessing these outcomes or de-emphasize the benefits associated with potential treatments of these symptoms. BV researchers and funders should legitimize the unique perspectives that women with BV bring to these issues by eliciting their values and priorities and factoring these into decisions about research funding, design, and implementation.

Conclusion

Excitement is growing around the clinical potential of microbiota transplantation, and VMT is on the cusp of entering the clinic. While microbiota transplantation itself has unique regulatory issues as a result of not fitting into the typical regulatory pathways, VMT specifically also has unique ethical issues related to the sex, race, and socioeconomic status of women comprising the target recipient population and donors. Here, we discussed the medical, regulatory, and ethical issues related to VMT, as well as design considerations for future clinical studies. We anticipate that the discussions herein will help accelerate the successful development of VMT.

Note

Dr. Ensign reports a patent "CVS transplantation for treatment of bacterial vaginosis" issued. The other authors have no conflicts to disclose.

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