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Probiotics: the Potential for a Live Microbicide to Prevent HIV

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INTRODUCTION

After early microbicide candidates failed in clinical trials to prevent HIV, recent news from the South African CAPRISA-004 study suggest that the new generation of vaginal microbicide gels using antiretroviral drugs like tenofovir could be effective and reduce HIV and herpes simplex virus (HSV)-2 acquisition.[1] This landmark study greatly energized the field and revived enthusiasm for the development of female-controlled products to prevent HIV acquisition in women, and if ongoing trials confirm the CAPRISA-004 results lead to a licensed product by 2014. [23]

While the field is getting closer to identifying an effective compound against vaginal HIV transmission, significant challenges remain, such as a sustained coitally-independent release of an effective drug, and a product formulation and administration that suits life circumstances of women in developing countries. Stigma continues to limit women's access to HIV prevention strategies, and many women underestimate their own risk of HIV infection[4]. Thus, a microbicide product perceived to improve overall vaginal health may decrease possible acceptability barriers that a single purpose product associated with HIV prevention may face.

In late May, the bi-annual global conference Microbicides 2010 held in Pittsburgh (USA) was attended by 1000 scientists and advocates, including more than 300 from Africa. The UCSF Bixby Center for Global Reproductive Health and the Consortium to Advance Multipurpose Innovations (CAMI) organized a Satellite Symposium titled 'Probiotics: the Potential for a Live Microbicide'. The event provided a platform for researchers working in related fields to educate the conference audience and to initiate a discussion with other scientists, donors, advocates, members of federal agencies and world bodies, and regulatory experts in order to accelerate the development of probiotics for HIV prevention.

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Meeting

Satellite Symposium titled 'Probiotics: the Potential for a Live Microbicide' at Microbicides 2010 in Pittsburgh, U.S.

A handful of small biotechnology companies and academic groups are working to develop a new generation of genetically enhanced probiotics by inserting genes which code for potent antiviral compounds into bacteria that naturally colonize the vagina. Once administered to the vagina, these next generation probiotics have the potential to serve as a sustained, self-replicating delivery system for antimicrobial compounds to combat reproductive tract infections, including HIV. The potential advantages of a probiotic microbicide continuously producing anti- HIV protein compounds *in situ* over conventional microbicide delivery systems such as gels and films include: i) periodic, possibly weekly or monthly replenishment vs. coitally dependent or daily dosing; ii) minimal disposal concerns, e.g. applicators; and iii) low-risk of developing HIV resistance in comparison to antiretroviral therapy commonly used in treatment, such as tenofovir.

However, the field to date has been hampered by a relative lack of interest among donors due to competing HIV prevention technologies under development, lack of clarity regarding the regulatory pathway for licensure and general sensitivity surrounding genetically modified organisms (GMO), and financial fall-out from the recent worldwide recession.

PROBIOTIC MICROBICIDES FOR HIV PREVENTION

The normal vaginal environment is dominated by self-replicating lactobacilli species that maintain an acidic pH and inhibit the growth of pathogens and subsequent infections. Research of probiotic lactobacilli to improve genital health has increased steadily over the last two decades. The first generation of probiotics uses selected human strains for the prevention of recurrent bacterial vaginosis (BV) and urinary tract infection (UTI) following standard antibiotic treatment. Over time, researchers in this field have utilized new diagnostic technology such as rDNA polymerase chain reaction, and improved product formulations and dosing regimens. Clinical trials have successfully demonstrated vaginal colonization with exogenous *Lactobacillus* strains and provided data on effectiveness against recurrent BV [5–8] and UTI [9]. Advances of these products will likely require testing of additional approaches, such as extending antibiotic treatment to more effectively destroy the bacterial biofilm, and overcoming the negative influence of semen exposure [10] and menstruation on the proportion of women colonized with the exogenous *Lactobacillus* strains.

The next generation of probiotics will be genetically engineered. Highly potent HIV inhibitors can be continuously produced by genetically enhanced self-renewing *Lactobacillus* bacteria that colonize the vaginal mucosa after periodical vaginal application.

The genetically modified *L. jensenii* 1153-1666 (MucoCept®) developed by Osel, Inc. follows this approach. Naturally occurring vaginal *Lactobacillus* strains were evaluated, and the *L. jensenii* 1153 was selected as the single best strain. Next, this strain was engineered to produce the potent HIV entry inhibitor Cyanovirin-N (CV-N). Lastly, Osel developed technology to preserve large quantities of MucoCept as a freeze-dried, stable powder of pharmaceutical grade quality.

The further development of next generation probiotics will require extensive pre-clinical and early clinical testing before their efficacy can be tested. In order to be effective, these

bacteria need to colonize the vagina in high concentrations in a large majority of women. In addition, the *in situ* protein expression and bioactivity, and the immunological responses of the host need to be carefully monitored. Experiments in 20 Chinese rhesus macaques showed consistent lactobacilli colonization of CV-N-expressing *L. jensenii* for up to 90 days, at high levels of 10^5–10^7 colony forming units (cfu) per swab.

In theory, since the CV-N protein is "foreign" and the *L. jensenii* is not native to the macaque, an antibody response to either the CV-N or to the lactobacilli is possible, rendering the molecule inactive as a microbicide. During regular tests using ELISA in macaques exposed for more than 6 months, no antibody response to either the recombinant CV-N or to *L. jensenii* has been found in blood or cervicovaginal lavage samples (personal communication, Qiang Xu). Furthermore, the strain was easily cleared by topical administration of azithromycin.

In a repeated low dose challenge model Chinese rhesus macaques receiving MucoCept in comparison to controls had a 62% reduction in the rate of simian HIV (SHIV) acquisition ($p=0.037$) [11]. Next steps for the development of this product include a pre-phase 1 clinical trial of MucoCept to evaluate colonization, clearance following antibiotic treatment and biocontainment in a small group of healthy volunteers. In addition, Osel is exploring MucoCept as a platform to co-express additional HIV inhibitors for as a multipurpose microbicide against HIV and other sexually transmitted infections (STIs).

Other groups of researchers are exploring similar concepts. ActoGenix, a Belgian company, is using genetically modified *Lactococcus lactis*, derived from the food industry, as a platform to deliver proteins such as the anti-inflammatory cytokine Interleukin-10 to down regulate inflammatory bowel disease and ulcerative colitis. A *L. lactis* producing Trefoil Factor 1 (TFF1) has been designed to prevent colitis [12] and oral mucositis [13], a debilitating and painful side effect of radio- and chemotherapy. Clinical phase 2 studies are under way for these products, and ActoGenix is also exploring *L. lactis* as a safe platform to deliver pro-insulin to treat juvenile diabetes.

Due to the mixed perception and consideration of testing genetically modified organisms (GMO) in different regions of the world and in order to increase donor support to develop these products, the field needs to educate key stakeholders including the regulatory agencies around the world. The regulatory approval process for probiotic drugs faces unique challenges. First, in contrast to probiotic foods, pharmaceutical grade drugs need to be produced in facilities complying with Good Manufacturing Practices (GMP). Second, drugs that are deliberately releasing GMO need to follow country-specific guidelines addressing biocontainment and eradication. Third, regulatory agencies in different countries may have unique requirements that need to be sufficiently and proactively addressed when planning for and designing future clinical studies.

While women in sub-Saharan Africa have the highest need for female-controlled HIV prevention technologies, women in other regions also require better prevention tools against HIV and other STIs. In tandem with the scientific development of these drugs, scientists and companies should concern themselves with the different contexts in which this technology

may be introduced. End-user communities need to be involved from the beginning in order to create awareness and ensure their support and input for the clinical research and the eventual marketing and distribution. In addition to potential users, other gate keepers like their male partners, community leaders and health care providers need to be engaged. Importantly, the daily realities of end-users such as storage, sanitary requirements, disposal options, and cost need to be considered to ensure that probiotic microbicides proven effective for HIV prevention will be used.

CONCLUSION

The CAPRISA-004 results restored enthusiasm for microbicides as a key technology to prevent HIV. In order to fulfill the promise of microbicides, research for additional antiretroviral compounds and delivery mechanisms needs to be stepped up. Women are waiting for safe, easy to use, inexpensive and efficacious technologies to help them prevent HIV and other STIs. Probiotics, as a potential live microbicide, offer significant advantages including their safety profile, and a simplified self-replicating drug delivery platform. In addition, probiotics could serve as a component of multipurpose prevention tools (MPTs) for sexual and reproductive health to prevent multiple adverse health outcomes simultaneously, including HIV, STIs, unplanned pregnancy, as well as other reproductive tract infections such as BV.

As a new technology, the development of enhanced probiotics as HIV prevention drugs faces complex and unique challenges including competition for financial support, regulatory hurdles, manufacturing and logistical barriers and effective branding and commercialization. To overcome these barriers it is critical to forge multidisciplinary alliances of scientists, advocates, funders, government agencies, regulators, health care providers and community of end-users. We will need to build strong alliances to create the momentum to successfully move live microbicides from the laboratory to the community.

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