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**VAGINAL *LACTOBACILLUS* BIOENGINEERED FOR MUCOSAL DELIVERY OF THE  
ANTI-HIV MOLECULES**

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In healthy women of childbearing age, vaginal mucosa is densely populated with lactobacilli. Depletion or disruption of the *Lactobacillus*-dominated microflora increases the incidence of bacterial vaginosis, urinary tract infections, yeast infections, and viral infections, such as HSV-2 and HIV. Here we report progress in the development of a novel, live microbicide. A human vaginal isolate of H<sub>2</sub>O<sub>2</sub>-producing *Lactobacillus jensenii* was engineered to secrete several different potent HIV inhibitors, N-terminally modified Cyanovirin-N (CV-N), single chain antibodies, RANTES and its analogs. For the HIV entry inhibitor CV-N (P51G), an expression cassette was constructed and stably integrated into the *Lactobacillus* chromosome by homologous recombination and shown to constitutively secrete high levels of full-length monomeric, biologically active CV-N by *L. jensenii*. *Lactobacillus*-produced CV-N potently inhibited infectivity of CCR5-tropic HIV<sub>BaL</sub> *in vitro* with an IC<sub>50</sub> near 1 nM. The engineered strain also exhibited highly increased inhibitory activity against *Gardnerella vaginalis* *in vitro*. This strain colonized the vaginal mucosa of Chinese rhesus macaques for over two months and produced bio-active CV-N *in situ*, following intra-vaginal administration of the bacteria. Vaginal biopsy analysis indicated there is no evidence of vaginal inflammation in macaques that are persistently colonized with this CV-N-expressing strain. The colonized lactobacilli can be completely cleared from the macaque vaginal flora following administration of a vaginal suppository of azithromycin. *Ex vivo* studies and studies to evaluate the efficacy of the CV-N-expressing strain against pathogens transmitted through mucosa in animal models are underway. Work has also begun to optimize bacterial manufacturing, preservation and dosage form.

