**Vaccine and SAMT-247 continuous released by intravaginal ring (IVR) decreased risk of SIV acquisition in macaque**

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**Abstract:**

**Background:** The HIV epidemic remains unabated in sub-Saharan Africa, particularly in adolescent women who have limited access to antiretroviral therapy. The deletion of the Env V1 region of the DNA/ALVAC/gp120 vaccine regimen has improved vaccine efficacy up to 65% in female macaques compared to RV144 trial. Furthermore, the protein zinc inhibitor SAMT-247 formulated as vaginal gel in combination with ΔV1 DNA/ALVAC/gp120/alum vaccine showed 92.7% vaccine efficacy. Since the use of vaginal gel is not user-friendly, we developed an intravaginal ring (IVR) that continuously releases SAMT-247 and combined it with the ΔV1 DNA/ALVAC/gp120/alum vaccine to replicate the vaccine efficacy observed in previous studies. We also aimed to investigate the *ex vivo* effects of SAMT-247 on the immune responses of both vaccinated and naïve animals.

**Methods:** Twelve macaques were vaccinated with the ΔV1 DNA/ALVAC/gp120/alum vaccines at weeks 0, 4, 8, and 12. In week 13, SAMT-247 IVR devices were inserted into the animals and replaced every 4 weeks. Ten naïve animals also received SAMT-247 IVR at the same time as the vaccinated animals, serving as controls. Mucosal samples and blood were collected before and 2 weeks after the insertion of SAMT-247 IVR to conduct immunological assays, including ADCC, efferocytosis, and flow cytometry, to measure cell frequencies and cytokine production, with a focus on immune responses linked to vaccine efficacy. All animals received up to 14 consecutive weekly intravaginal SIVmac251 challenges in the presence of SAMT-247 IVR until infection was confirmed.

**Results:** The historical vaccine-alone group showed a 65% reduction in the risk of virus acquisition (p=0.0074). Remarkably, the combination of the vaccine with SAMT-247 IVR led to an 84.2% decrease in virus acquisition compared to controls (p<0.0001). SAMT-247 IVR alone also significantly reduced the risk of SIV acquisition (p=0.048). However, the combination of the vaccine with SAMT-247 IVR was more effective than the SAMT-247 IVR alone (p=0.04). The inclusion of SAMT-247 IVR in the vaccine group increased the frequency of mucosal NKp44+ ILCs and gp120-induced IL-17+ NKp44+ ILCs, both of which were correlated with a reduced risk of SIV infection. SAMT-247 IVR also enhanced NK cell activity, which in turn boosted ADCC activity, further correlating with a decreased risk of SIV infection. Moreover, SAMT-247 increased the frequency of mucosal gp120-induced IL-10+ non-classical monocytes, CD73+ mDCs, and gp120-induced IL-10+ DC10, all of which were associated with reduced SIV acquisition. In contrast, while in naïve animals SAMT-247 IVR insertion increased mucosal NK cell frequency, PMA-induced CD107+ NK cells, CD73+ classical monocytes, CD73+ mDCs, and CD73+ DC10 cells, these were not linked to a reduced risk of infection. Therefore, SAMT-247 IVR act differently in vaccinated animals compared to naïve animals.

**Conclusions:** SAMT-247 increases the protective immune responses generated by vaccine, which leads to reduced risk of SIV infection. This data suggested controlled release of SAMT-247 by intravaginal ring, combined with the DNA/ALVAC/gp120/alum vaccine regimen, might result in an efficacious preventative strategy to end HIV endemic.