# SAMT-247 SYNERGIZES WITH ALVAC-BASED VACCINE TO PROTECT AGAINST SIVmac251 ACQUISITION

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## Background

Canarypox-based HIV vaccine platforms with alum have demonstrated modest efficacy in both macaques ($SIV_{mac251}$ model) (Vaccari, et al., 2016) and humans (RV144 trial) (Rerks-Ngarm, et al., 2009). The failure of the HVTN702 trial further emphasizes the importance of improving vaccines for HIV. Substituting the ALVAC-SIV prime with V1-deleted envelope DNA, and the protein boost with V1-deleted gp120 protein in the ALVAC based immunization regimen improved the vaccine efficacy to fifty-seven percent in male macaques (Silva de Castro, et al. 2021). The efficacy of ALVAC-based vaccine modalities is linked to the induction of anti-inflammatory monocyte innate immunity (Vaccari, et al. 2016; Vaccari, et al. 2018) together with V2-specific antibody-dependent cellular cytotoxicity (Silva de Castro, et al. 2021).

Topical microbicides provide another important strategy for preventing HIV transmission, particularly in females who are not only biologically more susceptible but may also be socially and culturally more vulnerable. The concept of combining vaccines with microbicides in order to provide better protection compared to either approach alone led us to consider SAMT-247 (S-acyl-2-mercaptobenzamide thioester), a new class of microbicide that targets the mutationally intolerant HIV nucleocapsid protein (NCp7) zinc fingers (Turpin, et al., 2008) rather than typical viral enzyme targets. As a result of acetylation of the cysteine sidechains of the NC by the microbicide, zinc is ejected and the native structure of the protein is lost along with the ability to bind viral RNA, leading to loss of multiple functions (Miller Jenkins, et al., 2005) including impaired Gag maturation and loss of infectious particles (Turpin, et al., 1996).

**Hypothesis**-Combining the DNA/ALVAC/gp120 based vaccine regimen with SAMT-247 microbicide will provide enhanced protection against HIV/SIV infection.

## Study design

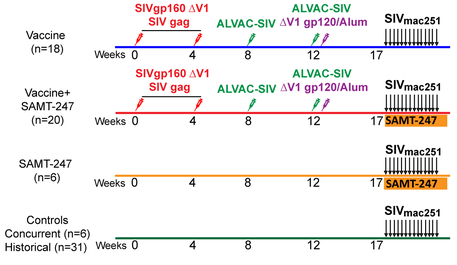



**Figure-1.** Fifty rhesus macaques were subdivided into four groups: vaccine, vaccine plus SAMT-247, SAMT-247, and controls. Thirty-eight animals were primed with DNA-SIVgp160 ΔV1+ SIV mac239 gag and boosted with ALVAC-SIV encoding env, gag and pol and ALVAC-SIV + ΔV1 gp120 protein in alum hydroxide at the indicated timepoints. Twelve animals remained naïve until SIV challenge. Beginning at week 17, protective efficacy against SIVmac251 was assessed by subjecting all animals to up to 14 weekly intravaginal viral exposures (arrows) in the presence or absence of SAMT-247 until infection was confirmed. Animals either received 0.8% SAMT-247 in HEC gel (n = 26) or HEC gel only (n = 24) 4 h prior to each low-dose SIVmac251 challenge. 31 historical controls that were intravaginally challenged with the same lot and dose of SIVmac251 were added to increase statistical power.

## Results

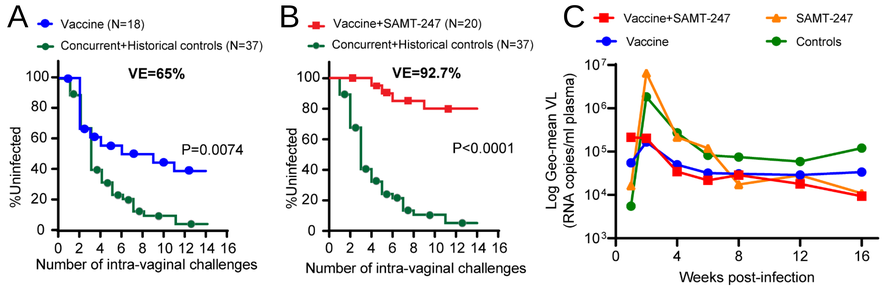



**Figure-2.** Significant protection in A) the vaccine group (vaccine efficacy of 65%) and B) the vaccine+SAMT-247 group (vaccine efficacy of 92.7%) compared with the concurrent+historical controls. The vaccine efficacy for vaccine group was 65% and for Vaccine+SAMT-247 was 92.7%. C) VL geometric means of all macaque groups over time. The peak viral load was significantly lower in the vaccine and vaccine-SAMT-247 groups compared to the microbicide only group. The control group maintained a comparatively higher geometric mean VL compared to the other macaque groups.

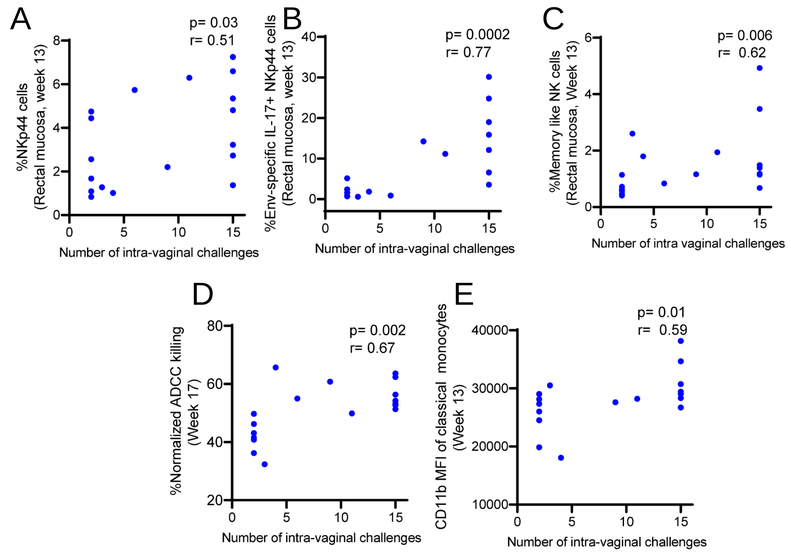



**Figure-3.** Evaluation of vaccine induced correlates of protection. Vaccine induced A) mucosal NKp44⁺ ILCs, B) mucosal NKp44⁺ IL-17⁺ ILCs, C) mucosal memory like NK cells, D) ADCC killing and E) Classical monocytes correlated with the number of challenges required for SIV infection. Here ● represents animals from the vaccine group.

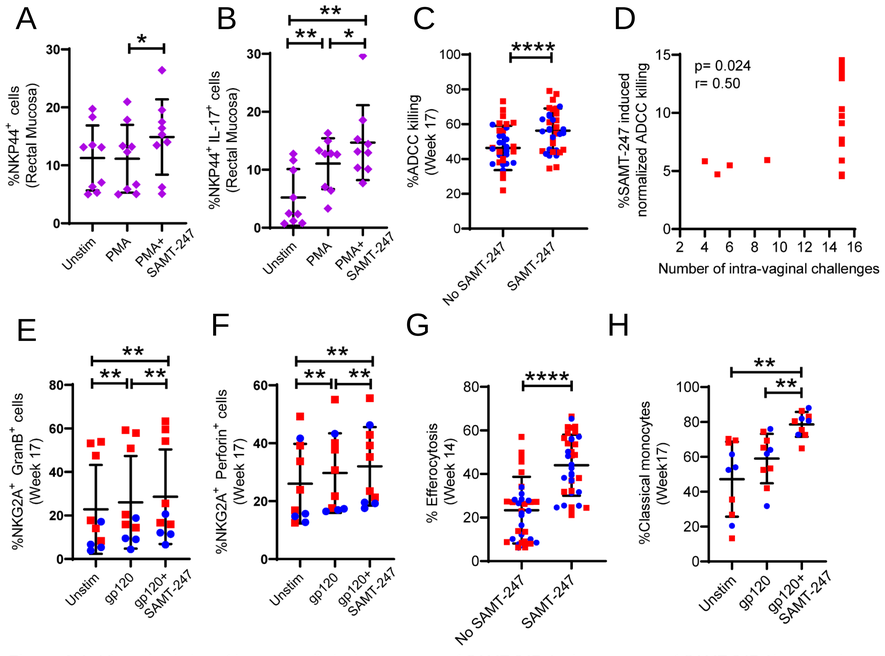
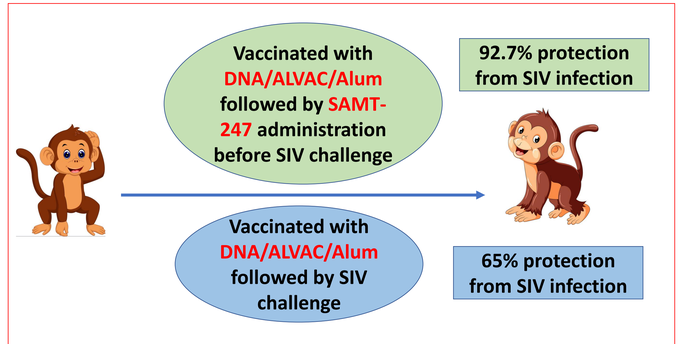



**Figure-4.** *In Vitro* stimulation of immune cells in the presence of SAMT-247. In the presence of SAMT-247 A) mucosal NKp44⁺ ILCs, and B) mucosal NKp44⁺ IL-17⁺ ILCs increase compared to unstimulated, or PMA stimulated cells. C) SAMT-247 increases ADCC killing and D) SAMT-247 induced ADCC killing correlated with the number of challenges required for SIV infection in the vaccine+SAMT-247 group. In the presence of SAMT-247 ADCC effector cells produce higher levels of E) granzyme B and F) perforin. G) The apoptotic cells generated by ADCC are cleared by efferocytosis and in the presence of SAMT-247 efferocytosis is also increased. F) The protective classical monocytes in blood also increases after SAMT-247 treatment. Here ● represents animals from vaccine group, ■ vaccine+SAMT-247 group and ◆ age-matched macaques from a separate study; 4 vaccinated against HIV and 5 naïve, all challenged with SHIV, rectal tissues obtained post challenge from 7 infected and 2 uninfected animals.

## Summary

- Modified DNA/ALVAC/ gp120 based vaccine showed a vaccine efficacy of 65%.
- In combination with SAMT-247 the vaccine efficacy increased to 92.7%.
- *In vitro* studies showed that SAMT-247 modulates the correlates of protection and helps to achieve a higher degree of protection against SIV challenge.




| Correlates with protection | Effect of SAMT-247 *in vitro* |
|---|---|
| • ADCC | ↑ |
| • Classical monocytes | ↑ |
| • Mucosal NKp44⁺ cells | ↑ |
| • Mucosal NKp44⁺ IL-17⁺ cells | ↑ |
| • Memory NKG2A⁺ cells | Not checked |