EFFICACY OF AD26/MVA + ENV + VESATOLIMOD THERAPEUTIC VACCINATION IN RHESUS MACAQUES

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BACKGROUND

- TLR7 agonists, such as GS-986, have previously been used in combination with both therapeutic SIV vaccination as well as with broadly neutralizing antibodies (bNAb) in SIV and SHIV infected rhesus macaques while on ART to reduce viral reservoirs and confer virologic control after ART discontinuation (1-2)
- Vesatolimod (GS-9620) is a close analog of GS-986 that similarly induces TLR7 signaling (3-5).
- In this study, we investigated an Ad26/MVA therapeutic vaccine in combination with repeated vesatolimod administration in 36 ART-suppressed rhesus macaques and included a SIV_{mac32E} Envelope gp140 (Env) boost with alum adjuvant to determine the therapeutic efficacy of both enhanced cellular and humoral immune responses via the addition of an Env protein immunogen.

METHODS

SIV-specific cellular immune responses post Ad26/MVA + Env with repeated Vesatolimod administration between each Env boost were determined via ELISpot and intracellular cytokine secretion assays. Humoral responses were assessed by SIV Env-specific IgG ELISA. Intact replication-competent proviruses were measured with a digital droplet PCR-based intact proviral DNA assay (IPDA) with SIV-specific primer sets. Vesatolimod efficacy was determined with serum cytokine profiling and expression of peripheral activation markers (i.e. CD69) using multiparameter flow cytometry. Therapeutic efficacy of the vaccine regimen was assessed longitudinally up to 198 days following ART interruption by measuring plasma viral loads via qRT-PCR.

RESULTS

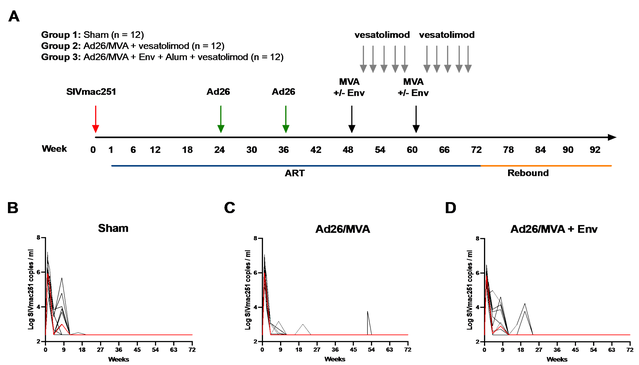

Figure 1: Ad26/MVA + Env protein subunit boost therapeutic vaccine strategy with vesatolimod treatment. (A) Therapeutic vaccine study design. (B-D) Log transformed plasma SIV_{mac251} viral loads (gag copies / ml plasma) from onset of infection to week 72 post-infection. Limit of detection (LOD) for the SIV viral load qRT-PCR assay used in the study was > 250 *gag* copies / ml plasma. Red lines denote median values across all time points.

Developing an intervention that results in virologic control following discontinuation of antiretroviral therapy (ART) is a major objective of HIV-1 cure research. In this study, we investigated the therapeutic efficacy of a vaccine consisting of adenovirus serotype 26 (Ad26) and modified vaccinia Ankara (MVA) with or without an SIV Envelope (Env) gp140 protein with alum adjuvant in combination with the TLR7 agonist vesatolimod (GS-9620) in 36 ART-suppressed, SIVmac251-infected rhesus macaques. Ad26/MVA therapeutic vaccination led to robust humoral and cellular immune responses, and the Env protein boost increased antibody responses. Following discontinuation of ART, virologic control was observed in 5/12 animals in each vaccine group, compared with 0/12 animals in the sham control group. These data demonstrate therapeutic efficacy of Ad26/MVA vaccination with vesatolimod but no clear additional benefit of adding an Env protein boost. SIV-specific cellular immune responses correlated with virologic control. Our findings show partial efficacy of therapeutic vaccination following ART discontinuation in rhesus macaques.

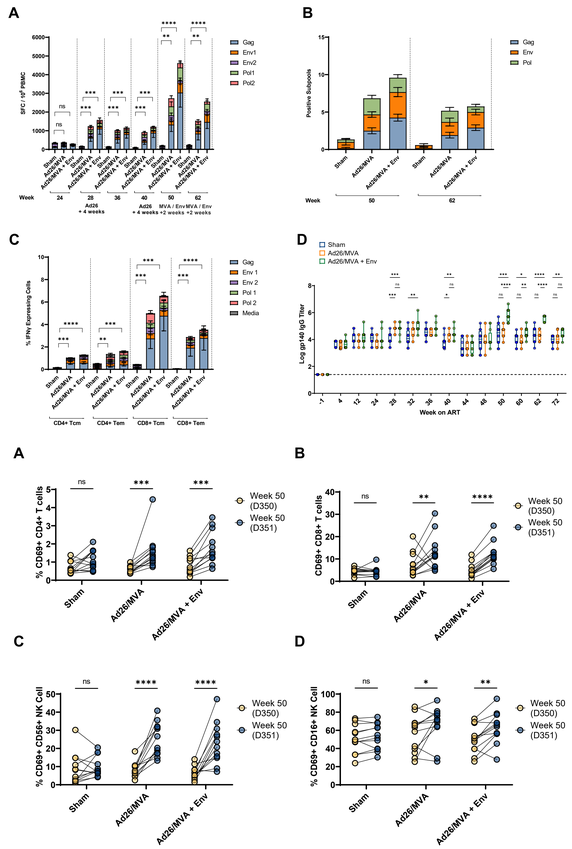

Figure 3: Cellular activation following vesatolimod administration. (A-H) T and NK cell activation in both sham and vaccinated groups as measured by increase in cell surface CD69+ expression one day following vesatolimod administration at week 50 post-infection in CD4+ T cells (A), CD8+ T cells (B), CD56+ NK Cells (C), and CD16+ CD56- NK Cells (D). Statistical significance determined from two-way ANOVA with Bonferroni correction for multiple comparisons. * < 0.05, ** < 0.01, *** < 0.001, **** <0.0001. Red lines denote median values.

Figure 2: Vaccine-elicited cellular and humoral immune responses following therapeutic vaccination and vesatolimod treatment. (A) SIV-specific cellular immunity against Gag, Env, and Pol peptide subpools determined from IFNγ ELISpot assays performed on PBMCs sampled during Ad26/MVA and Ad26/MVA + Env therapeutic vaccination. Data shown as Spot Forming Cells (SFCs) per million cells. P values were derived from a Kruskal-Wallis test with Dunn's correction for multiple comparisons of summed SFC counts for each treatment group. * < 0.05, ** < 0.01, *** < 0.001, **** <0.0001. (B) Total immune breath of vaccine-induced SIV-specific IFNγ production quantified as the summation of total Gag, Env, and Pol subpools. (C) Flow cytometric assessment of CD4+ and CD8+ intracellular IFNγ secretion at week 62 post-infection following a 9-hour stimulation with SIV_{mac239} Gag, Env, and Pol peptides. P values were derived from a Kruskal-Wallis test with Dunn's correction for multiple comparisons from summed fractions of IFNγ positive cells for each treatment group. * < 0.05, ** < 0.01, *** < 0.001, **** <0.0001. (D) SIV Env IgG titers measured by ELISA between 4 weeks prior to SIV_{mac251} infection (-4 weeks) and 72 weeks post-infection. Data shown as a box and whisker plot of log transformed values displaying the max and min values, median, and quartiles. P values were derived from a two-way ANOVA with Bonferroni correction for multiple comparisons. * < 0.05, ** < 0.01, *** < 0.001, **** <0.0001.

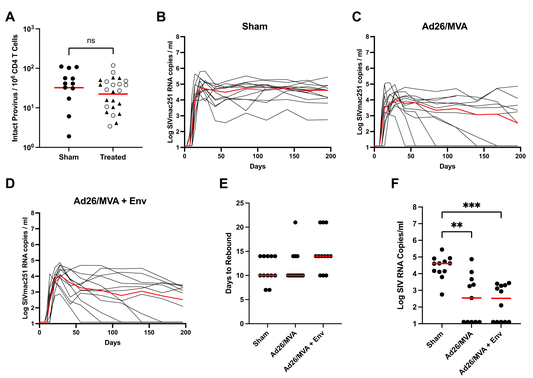

Figure 4: Figure 4: Control of rebound viremia following antiretroviral therapy interruption. (A) Intact proviruses per million CD4+ T cells quantified via an SIV-optimized digital-droplet PCR based Intact Proviral DNA Assay (IPDA) measured at week 72 post-infection, directly before ART interruption. Treatment groups were aggregated into a single group and compared to sham controls. Solid Triangles are animals in the Ad26/MVA treatment group and open circles are animals from the Ad26/MVA + Env treatment group. Red lines show the geometric mean. Statistical significance was derived from a nonparametric Mann-Whitney U Test. (B-D) Plasma viral loads (in log SIV_{mac251} *gag* copies / ml plasma) for each study group 198 days following ART interruption at week 72 post-infection. Red curves indicate median values. (E) Number of days until rebound infection was detected after ART interruption at week 72 post-infection. Red lines indicated median values. Statistical significance was determined from a Kruskal-Wallis test with Dunn's correction for multiple comparisons. (F) Plasma viral loads detected at day 198 post-ART interruption, for each treatment group and sham animals. Red lines denote median values. Statistical significance was determined from a Kruskal-Wallis test with Dunn's correction for multiple comparisons. * < 0.05, ** < 0.01, *** < 0.001, **** <0.0001.

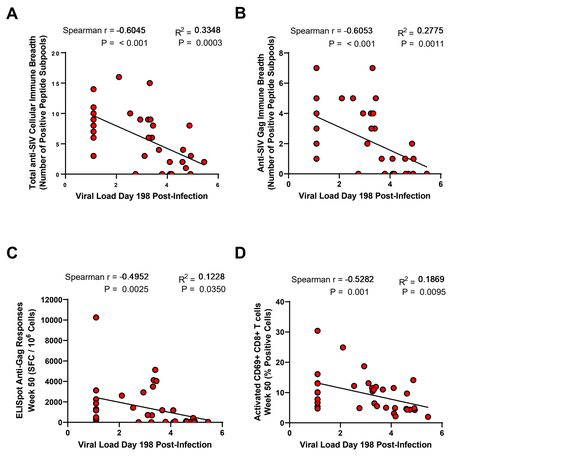

Figure 5: Immune correlates of virologic control following antiretroviral therapy interruption. (A-D) Spearman correlation plots between total cellular anti-SIV immune breadth as determined by the number of positive subpools detected via IFNγ ELISpot (A), the total anti-Gag Immune breadth as determined by the number of Gag-positive subpools detected via IFNγ ELISpot (B), anti-Gag IFNγ ELISpot responses quantified as SFC / 10^6 cells (C), frequency of activated (i.e. CD69+) anti-viral CD8+ T cells at week 50 post-infection (D), and the viral load determined at day 198 after ART interruption. Spearman r coefficients and P values are displayed above each correlation plot. R^2 values and best fit lines are also displayed following an additional linear regression analysis.

CONCLUSIONS

The data from this study showed that Ad26/MVA + SIV Env with alum adjuvant therapeutic vaccination and vesatolimod administration led to enhanced SIV-specific cellular immune responses when compared to the Ad26/MVA regimen alone. IgG Titer at weeks 50, 62, and 72 did not significantly correlate with viral loads at Day 198. Overall, these results provide a rationale for future studies involving Ad26/MVA therapeutic vaccine regimens as a potential HIV-1 functional cure.

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