

INTRAVAGINAL APPLICATION OF A NOVEL ANTIVIRAL MIXTURE INHIBITS VAGINAL TRACT MmuPV-1 INFECTION IN MICE

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Background:

Medicinal plants are a rich source of bioactive compounds. Many act as phytoalexins to defend against pathogens. Historically, they laid the foundation for drug discovery. We have created phytochemical mixtures targeting HPV-positive cancer cell lines, effectively suppressing HPV E6/E7 proteins and modulating immune response genes in vitro. These mixtures were incorporated into an intravaginal cream and further optimized using the MmuPV1 vaginal papilloma mouse model. This clinically relevant model, using immunocompetent animals, mimics mucosal HPV infections and serves as an ideal system to test local antiviral drug delivery.

Antiviral Compound Discovery & Synergistic Formulation Development

- We previously identified three classes of phytochemicals (curcuminoids, catechins, and diphenylethylenes) that eliminate HPV (+) cancer cell lines both in vitro and in murine cancer models (1,2)
- We hypothesized that medicinal plants would contain additional derivatives. Thus, we used the structural motifs of these small molecules to screen for additional phytochemicals.
- Synergistic formulations were created using combinatorial drug testing in a panel of HPV (+) cell lines (3). These compositions were developed into a topical micro-emulsion suitable for intravaginal delivery.

STUDY OBJECTIVE:

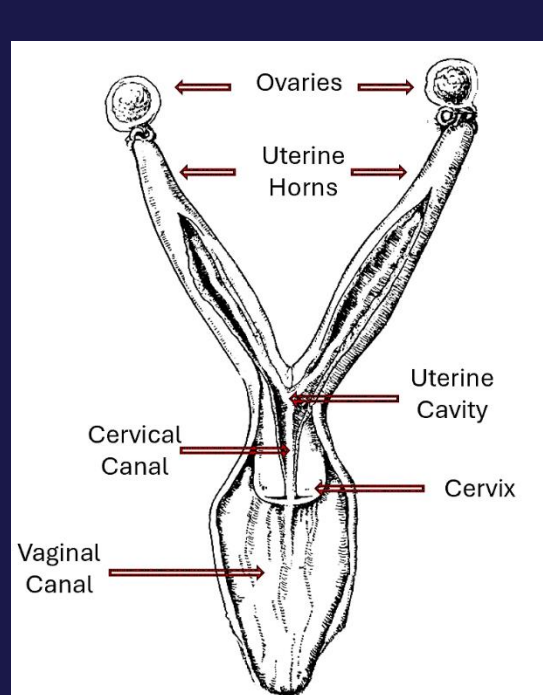
This study evaluates the in vivo anti-viral activity of formulated topical mixtures using the CRPV/rabbit model. Various synergistic combinations are tested, with efficacy assessed by the number, growth rate, and size of papillomas over a 5-week treatment and a 3-week post-treatment observation period.

METHODS

MmuPV1 Vaginal Papilloma Mouse Model

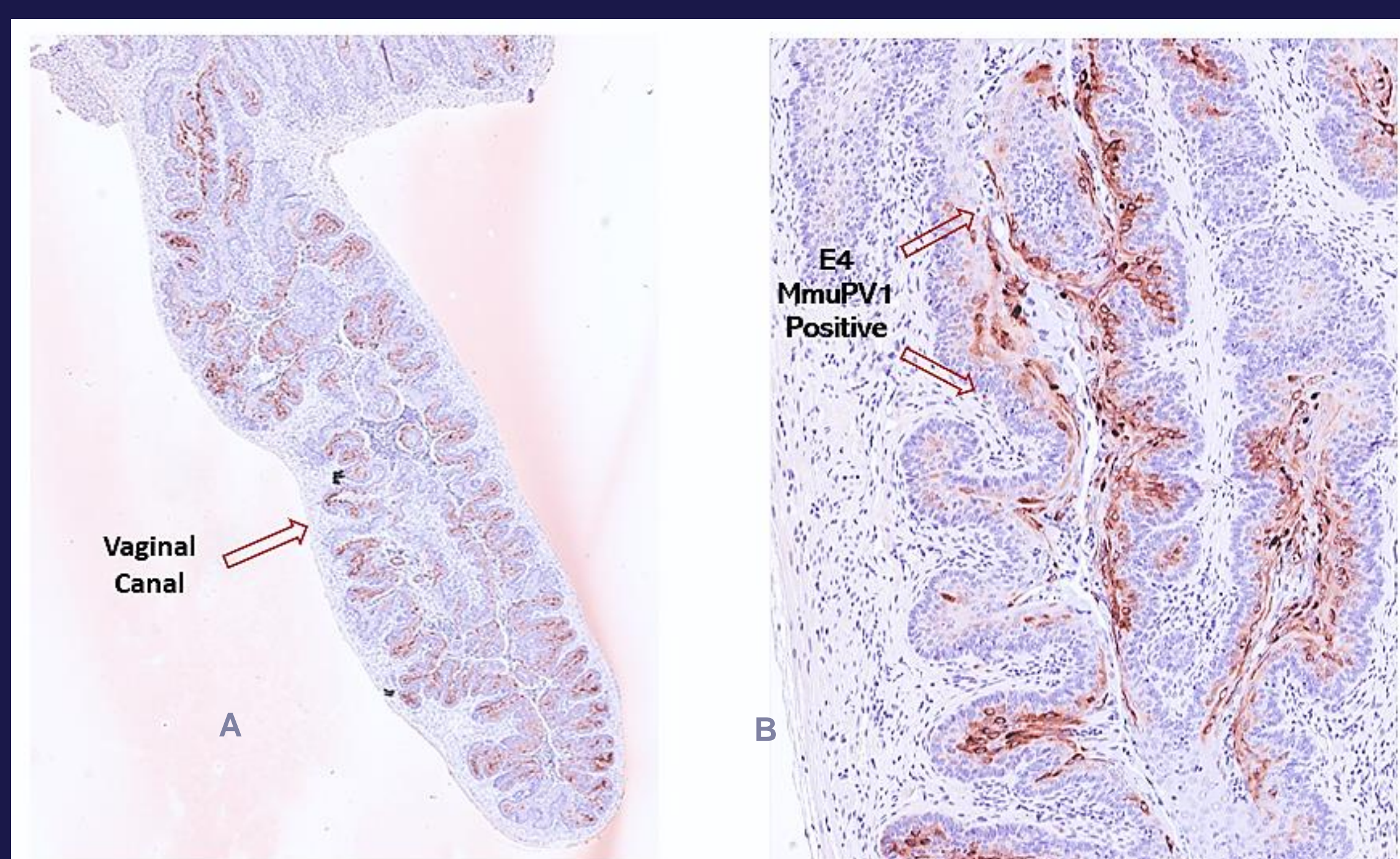
- **Inoculation:** 25 female heterozygous Nu/J mice inoculated with MmuPV-1 at vaginal sites
- **Treatment:** 25 µl of drug applied intravaginally with soft pipette tip; 5 times/week for 5 weeks
- **Groups:** Drug Mixtures Groups A-C; Group D Positive Control & Group E as placebo control
- **Monitoring:** Vaginal lavages to assess viral DNA copy number weeks 3 & 4
- **Endpoint:** Histology of vaginal tissues for papilloma morphology and toxicity

Female Mouse Reproductive Tract



Drug	Dosing Regimen (M,T,W,T,F)	Number of Mice	Intravaginal Dose
Mixture			
Group A	TW-SD1	5	25 µl
Group B	T-SD1	5	25 µl
Group C	TW-SD2	5	25 µl
Group D	Cidofovir	5	25 µl
Group E	Vehicle	5	25 µl

Pathologic Examination of MmuPV1 Infected Mice at the End of Treatment: Vehicle Control



Panel A: Diffuse brown staining indicates MmuPV1-positive areas detected using an anti-E4 probe. E4 expression serves as a marker of active viral replication.

Panel B: Extensive E4-positive MmuPV1 areas detected in the vaginal epithelium. However, there is no stromal immune cell infiltration observed.

Figure 1. Vehicle Treated Mice (Group E) after 5 weeks of treatment (study week 7)

Histologic Analysis of MmuPV1-Infected Mice Post-Treatment: Group C (T-SD2)

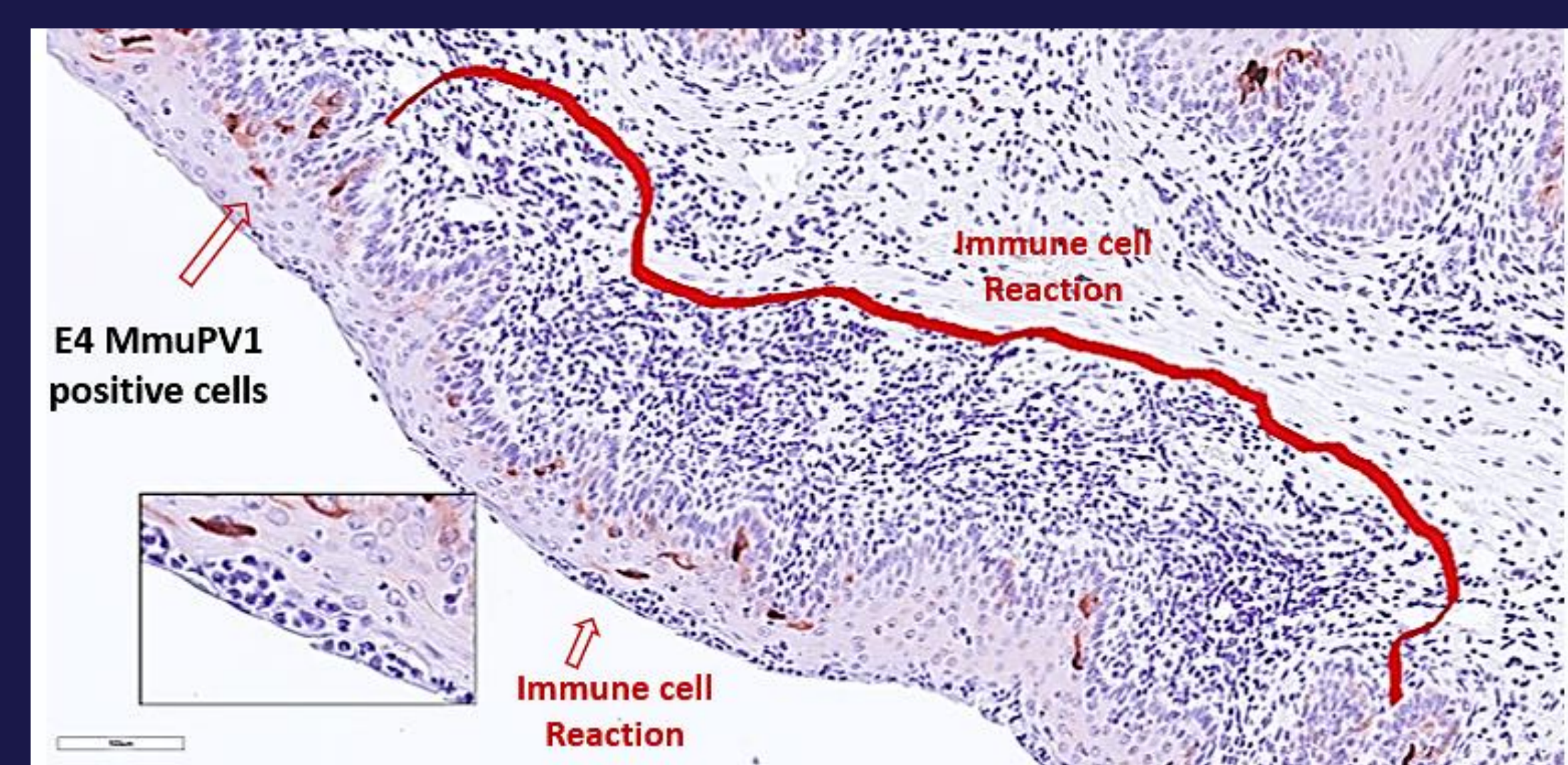


Figure 2. Reduced MmuPV1 (+) areas (brown staining) in T-SD2 treated samples compared to controls. A marked decrease in MmuPV1 (+) epithelial cells is accompanied by intense antiviral immune cell infiltration in the stromal, forming inflammatory cell nests absent in control samples.

Effect of Mixture INV-015 (Group D) on Papilloma Size at Week 10

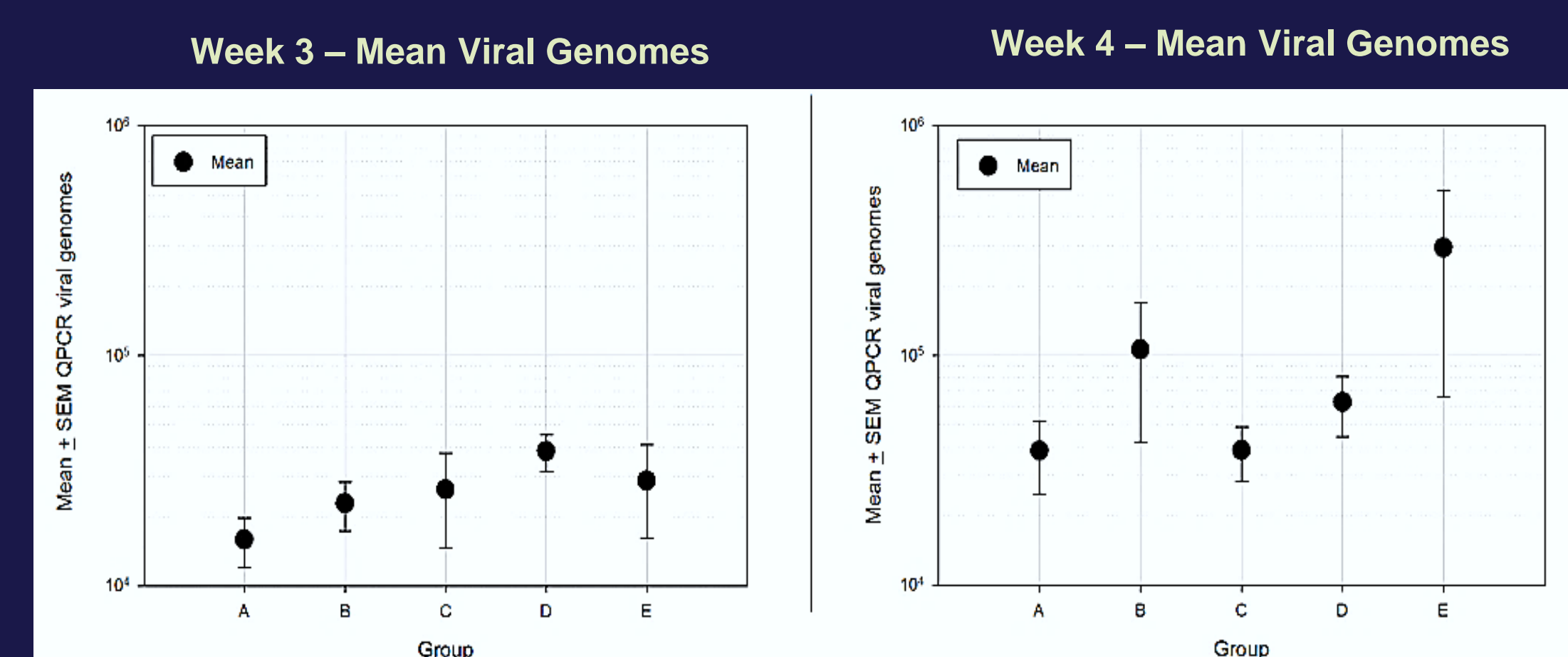


Figure 3. Comparison of Mean ± SEM QPCR viral genomes at Weeks 3 and 4 across treatment groups (A-E). Groups A and C (treated with TW-SD1 and T-SD2, respectively) showed a trend toward reduced viral loads compared to Group E (vehicle), although the reduction was not statistically significant. The positive control Group D (Cidofovir) also did not show an effect.

Fold change in Q-PCR signal (week 3 to week 4)

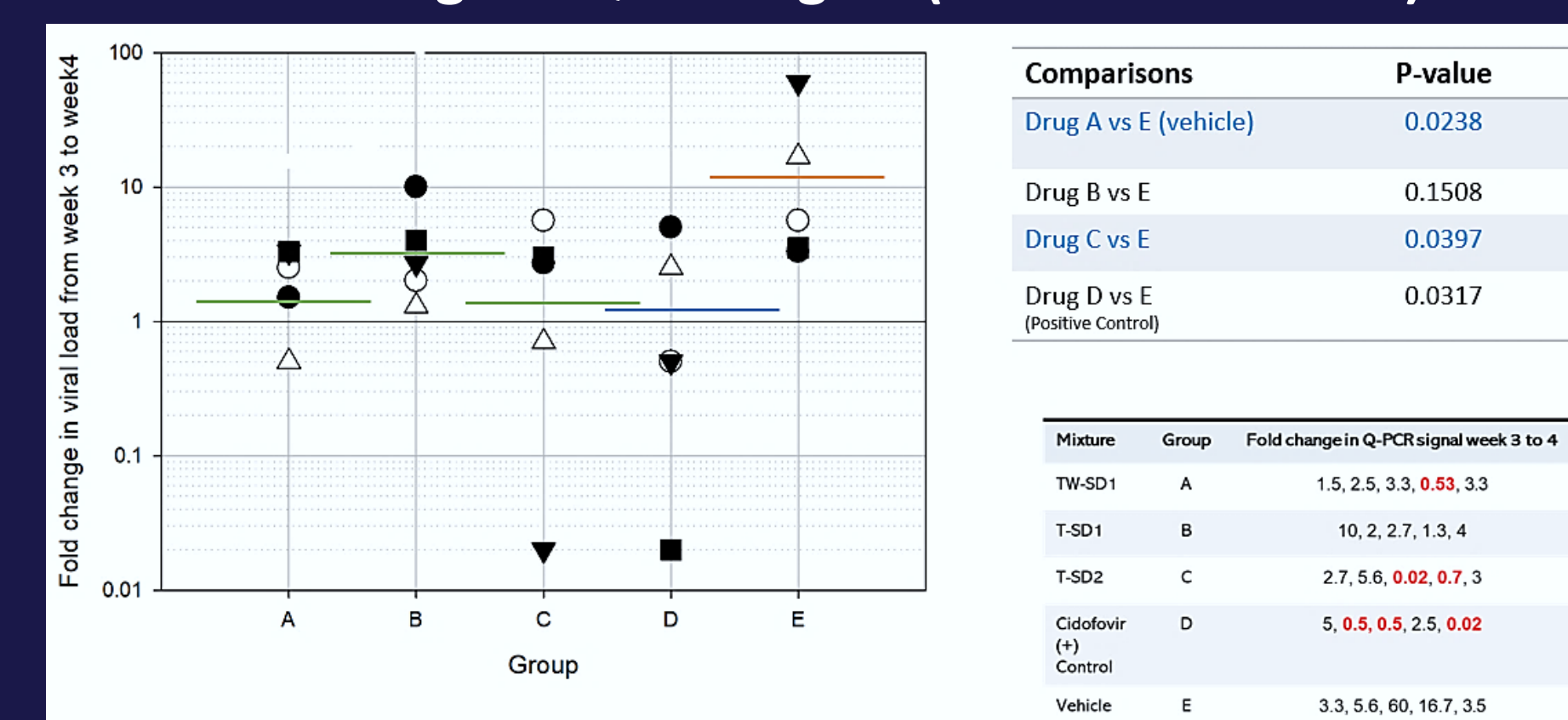


Figure 4. Comparison of fold change in viral load from Week 3 to Week 4 across treatment groups. Groups A (TW-SD1) & Group C (T-SD2) showed significant reductions in fold change compared to controls (Group E), p-values < 0.05. The positive control, Group D (Cidofovir), also demonstrated a significant reduction (p = 0.0317). While absolute viral load levels did not differ statistically between groups, these findings highlight the efficacy of the treatments in reducing viral replication rates.

CONCLUSION

We utilized a clinically relevant model that mimics mucosal HPV infections, which has been used to enhance our understanding of HPV pathogenesis. Our study validate the intravaginal drug delivery approach, demonstrating significant antiviral activity. Notable reductions in viral load were observed between Weeks 3 and 4 via vaginal lavage. Tissue analysis showed E4 protein inhibition—an indicator of viral replication. Treatment groups demonstrate an intense immune cell infiltration, not seen in control. These findings suggest that the formulations not only suppress viral replication but also enhance immune responses, providing a strong foundation for advancing these formulations into clinical trials for high-risk mucosal HPV infections.

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REFERENCES

- Mukherjee S, et al. Unique synergistic formulation of curcumin, epicatechin gallate and resveratrol, TriCurin, suppresses HPV E6, eliminates HPV+ cancer cells, and inhibits tumor progression. *Oncotarget*. 2017 Mar 29;8(37):60904-60916
- Piao L, et al. "TriCurin, a novel formulation of curcumin, epicatechin gallate, and resveratrol, inhibits the tumorigenicity of HPV-positive head and neck squamous cell carcinoma". *Oncotarget*. 2016 Jul 16
- PCT/US24/37345, "DIPHENYLETHYLENE COMPOUNDS AND COMPOSITIONS THEREOF."