

DEMONSTRATION OF EFFICACY OF A NOVEL TOPICAL ANTIVIRAL FOR TREATMENT IN CRPV-INFECTED RABBITS: A MODEL FOR HIGH-RISK HPV

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Background:

Despite advances, high-risk HPV infection has remained incurable. Given the virus's complex life cycle, successful therapy is postulated to require both antiviral & immunomodulatory properties. For this reason, we screened medicinal plants to identify three polyphenol classes with diverse activities. Synergistic mixtures eliminate HPV (+) cancer cell lines, inhibit HPV E6/E7 proteins, and induce p53/RB protein expression. Compounds were formulated into intravaginal creams and tested in a MmuPV1 mouse vaginal papilloma model. The most potent compounds have been further optimized and evaluated in the CRPV rabbit model.

The Development of an Anti-HPV Pharmaceutical Compositions

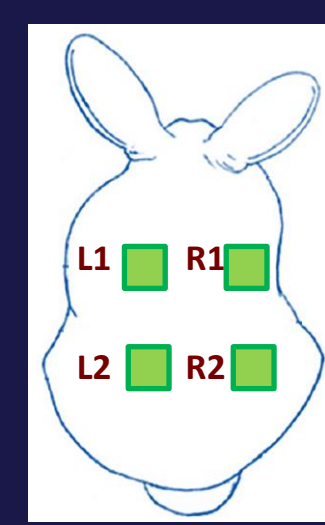
- We previously reported that three phytochemicals (curcuminoids, epigallocatechin & resveratrol), eliminate HPV (+) cancer cell lines both in vitro & murine cancer models (1,2)
- Transcriptome analysis of cells treated with the initial mixture (TriCurin) revealed significant gene expression changes, primarily linked to p53-dependent cell cycle arrest & modulation of cytokine pathways (3)
- During drug optimization, it was discovered that natural analogues of resveratrol alone and in combination with TriCurin produce enhanced in vitro effects, creating complex mixtures of 4-6 polyphenols (4).

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

In Vivo Testing : CRPV/Rabbit Model



- Mixtures evaluated via the NIAID/NIH In Vivo Drug Testing Program conducted at Penn State

- Rabbits infected cutaneously with the wild-type cottontail rabbit papillomavirus (CRPV) & mE8-CRPV

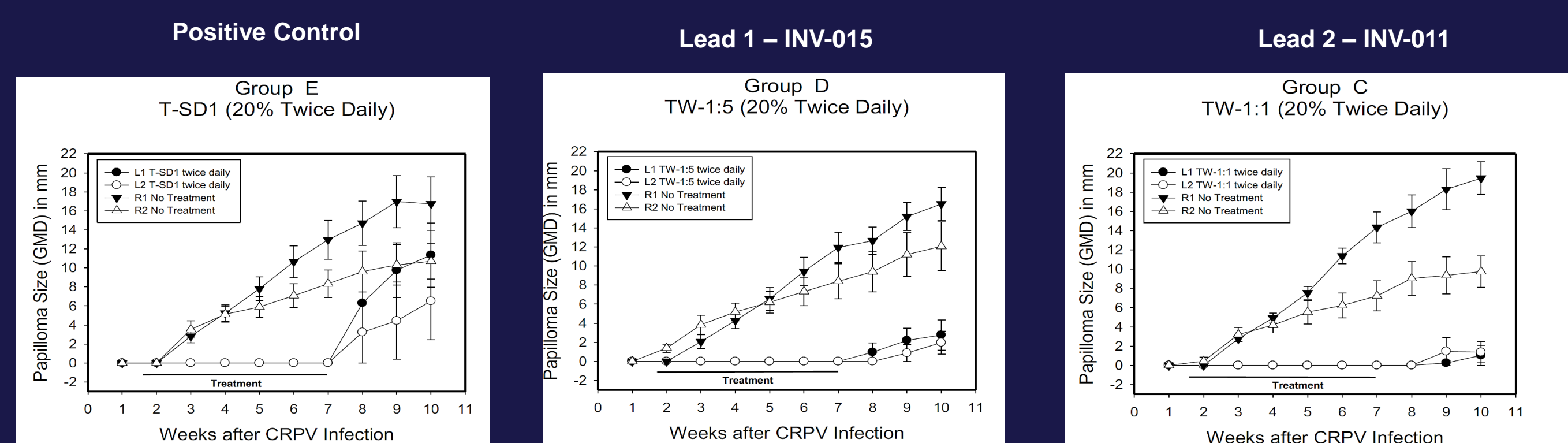
- Topical drug applied for 5 weeks

Topical Drug	Dosing Regimen (M,T,W,T,F)
	Mixture
Group A	TW-SD1
Group B	TW-SD2
Group C	INV-1:1
Group D	INV-1:5
Group E	Positive Control TriCurin-Pinostilbene (abbreviate T-SD1)

Virus	Treatment	Untreated
CRPV	L1	R1
mE8CRPV	R2	R2

CRPV = Cottontail Rabbit Papillomavirus
mE8-CRPV = mutant CRPV slower growing

Effects of Topical Antiviral Drugs on Papilloma size Over 10 Week study Period



T-SD1 Treatment Efficacy: No cures

- Wild-type CRPV (L1 vs. L2) 30.6% ↓ at 10 weeks
- Mutant CRPV (R1 vs. R2): 38.4% ↓ at 10 weeks

INV-015 Treatment Efficacy:

- Wild-type CRPV (L1 vs. L2) 40% lesions cured • remaining had 82 % reduction in size
- Mutant CRPV (R1 vs. R2): 60% lesions cured • remaining had a 81.9% reduction

INV-015 Treatment Efficacy:

- Wild-type CRPV (L1 vs. L2) 75 % lesions cured • remaining had 92 % reduction in size
- Mutant CRPV (R1 vs. R2): 50 % lesions cured • remaining had a 85.7% reduction

Figure 1. Mean ± SEM of Geometric mean diameter (GMD) measurements of CRPV-induced skin papillomas from rabbits (Group C, D, E). Papillomas (4 sites) were induced on the back skin with 5 µg of wt-CRPV DNA (●, ▼) or 25 µl mE8-CRPV virion stock (○, △). Topical treatments included TW-SD1 (TriCurin-Pinostilbene, which served as the positive control), INV-015, & INV-011 all at 20% twice-daily (●, ○), and untreated (▼, △). Each symbol represents the Mean ± SEM of the GMDs of the weekly measurements.

Papilloma Cure Rates in Rabbits by Treatment Group

Group	Mixture Name	Rabbits with Cures wild-type CRPV warts	Rabbits with Cures mutant CRPV warts
Group E Positive Control	T-SD1 (TriCurin-Pinostilbene)	0/5 (0%)	0/5 (0%)
Group D Lead #1	INV-015	2/5 (40%)	3/5 (60%)
Group C Lead #2	INV-011	3/4 (75%)	2/4 (50%)

Figure 2. All mixtures achieved 100% suppression of papillomas during therapy. Significant cures were observed post-treatment in lead mixtures INV-015 & INV-011. Lesions not cured were significantly smaller (Figure 1) demonstrating strong efficacy in both wild-type and mutant CRPV warts.

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

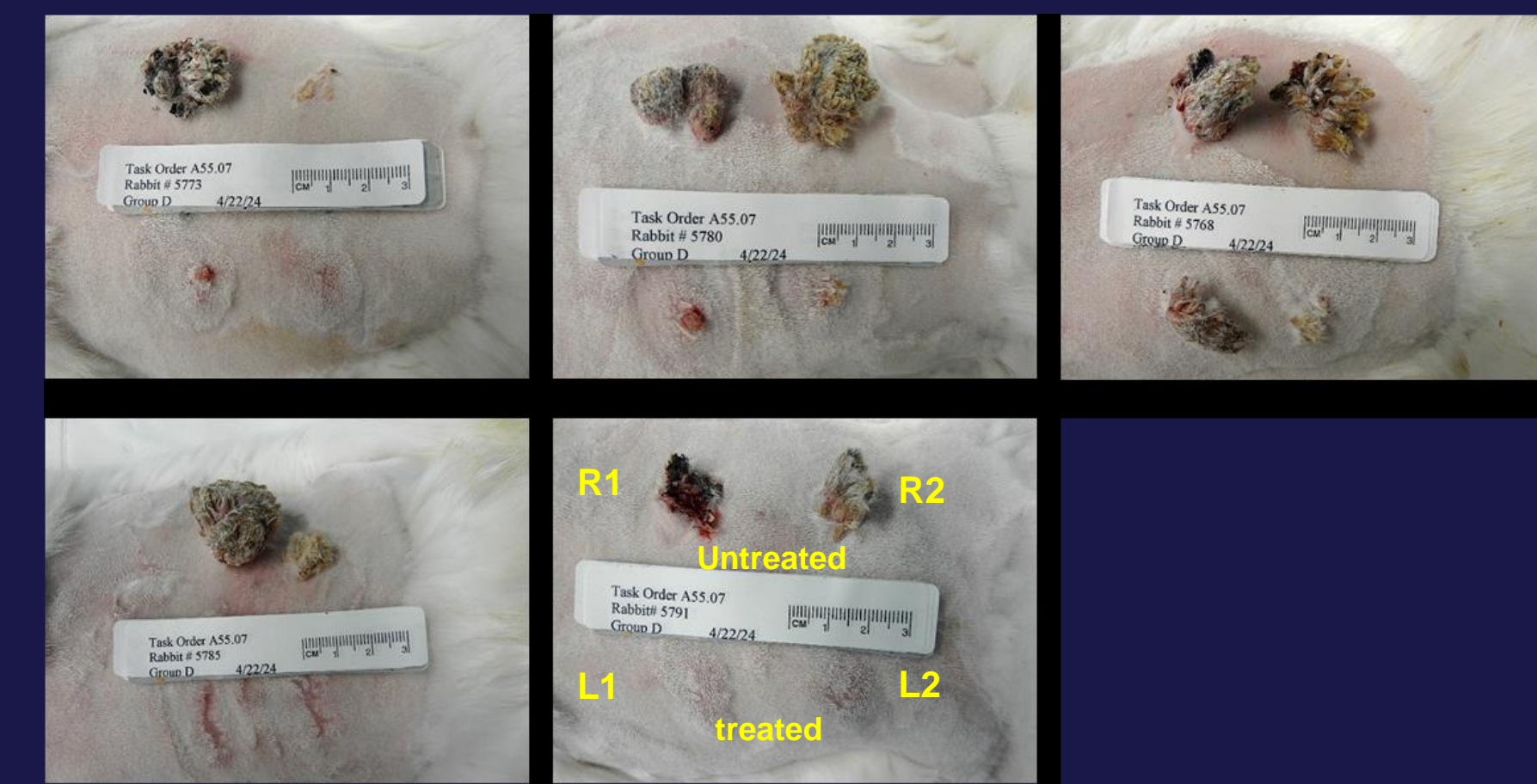


Figure 3. Images after 5 weeks of treatment and 3 weeks of observation. INV-015 demonstrated a strong antiviral effect with 40% lesion clearance in wild-type CRPV (L1 vs. L2) and 60% in mutant CRPV (R1 vs. R2). Residual lesions showed substantial size reductions. Normal appearing epithelium had no or minimal redness

Effect of Mixture INV-011 (Group C) on Papilloma Size at Week 10

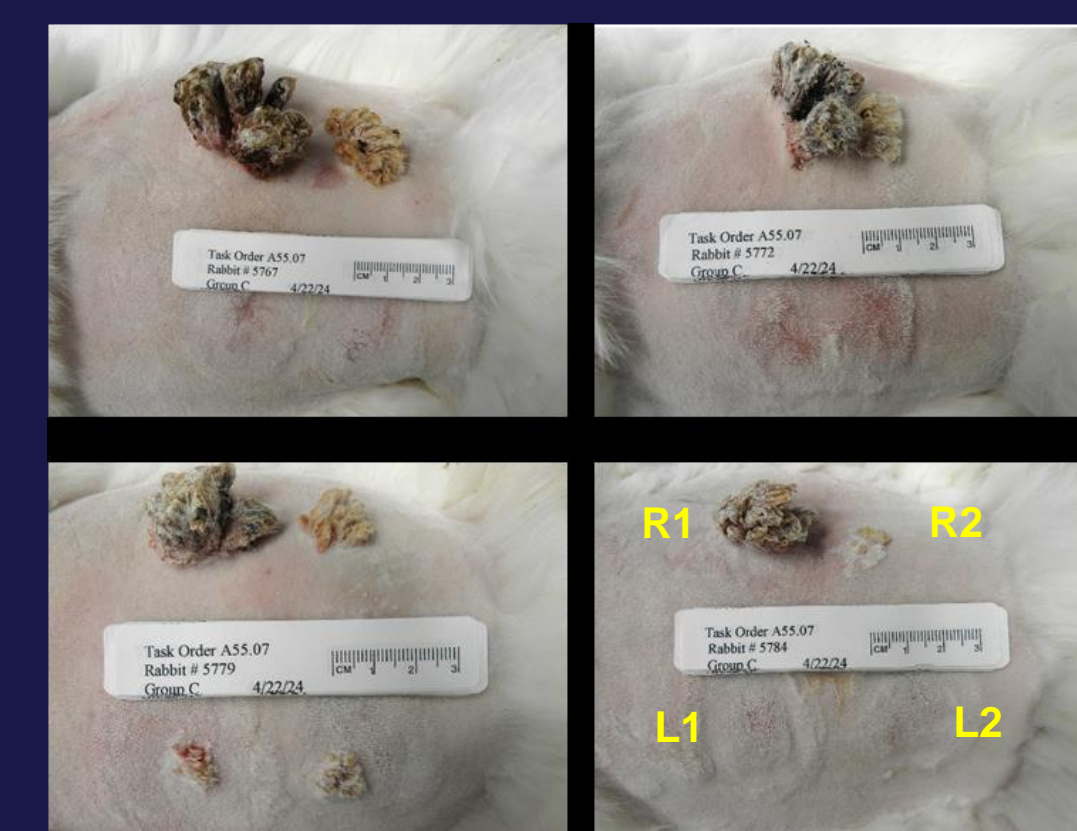


Figure 4. Images after 5 weeks of treatment & 3 weeks of observation. INV-011 demonstrated significant antiviral effects, 75% clearance of wild-type CRPV (L1 vs. L2) and 40% in mutant CRPV (R1 vs. R2).

CONCLUSION

The CRPV/rabbit model is known for its homology to high-risk HPVs serves as a gold standard for validating antivirals, and was instrumental in anti-HPV vaccine development. Two mixture achieved significant cures and reduced remaining papilloma sizes, each demonstrating slightly different effects on wild-type CRPV and the mutant mE8 virus. Our drug development approach was accomplished by optimization across mouse and rabbit models, providing compelling preclinical efficacy data. This establishes a strong foundation for advancement toward clinical trial testing.

Acknowledgment: Inovene is thankful for the invaluable support of the National Institute of Allergy and Infectious Diseases (NIAID/NIH) for providing non-clinical and pre-clinical services that contributed to this study.

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