

Introduction

With advances in understanding cancer-driving mechanisms, personalized and targeted therapies have become increasingly important. Oncolytic viruses (OVs) represent a promising class of therapeutics that enable selective delivery of vaccinia virus to tumors via intravenous administration. Armed vaccinia viruses can convert tumors into localized “anti-tumor drug factories,” secreting cytokines and chemokines that promote tumor cell destruction while minimizing systemic exposure and long-term adverse effects.

This study evaluated the potential toxicity of an oncolytic vaccinia virus following a single intravenous dose in LVG golden hamsters bearing Hap-T1 syngeneic pancreatic tumors.

Methods

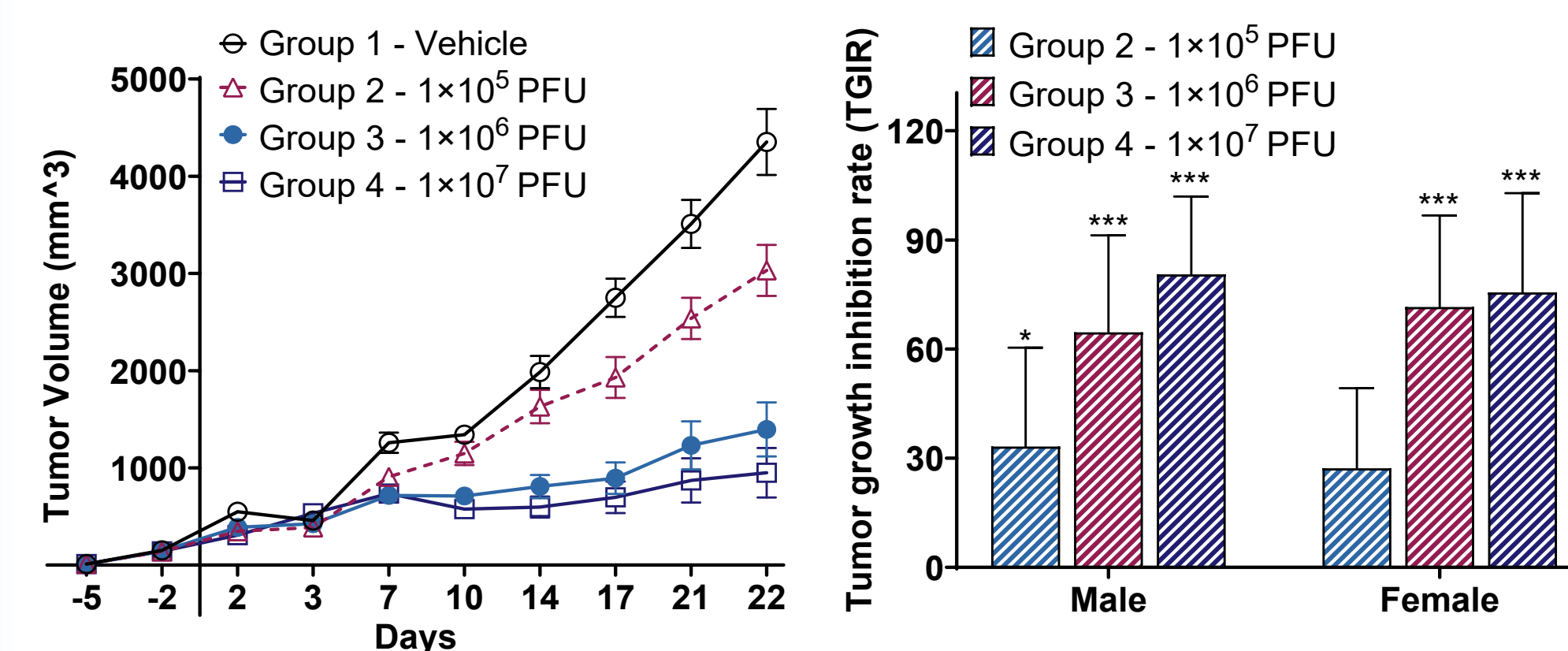
- Tumor model: Hap-T1 cells (5×10^7 cells/mL) were subcutaneously inoculated into the flank.
- Doses: 1×10^5 , 1×10^6 , and 1×10^7 PFU/animal (i.v.)
- Observation period: 21 days
- Parameters: Clinical signs, body weight, food intake, tumor growth, clinical chemistry, organ weight, histopathology, TK, biodistribution, neutralizing antibodies, and cytokines.

Results and Conclusion

- Tumor Inhibition: Tumor volume was significantly reduced in a dose-dependent manner (see Figure 1).
- TK/Neutralizing Antibody: Dose-dependent exposure declining over time; 100% neutralizing antibody positivity by Day 22 (see Figure 2).
- Biodistribution and Shedding: Virus mainly detected in adrenal glands and ovaries (see Figure 3).
- Survival/Tolerability: All animals survived to termination. Mild decreases in body weight and food intake at $\geq 1 \times 10^6$ PFU on Day 8 (see Figure 4). Minimal adrenal cortical necrosis/inflammation (non-adverse) at higher doses (data not shown). No abnormal cytokine changes (see Figure 5).
- A single intravenous dose of the oncolytic vaccinia virus demonstrated a NOAEL of 1×10^7 PFU/animal in LVG golden hamsters. These findings indicate a favorable safety profile and durable anti-tumor efficacy.

1

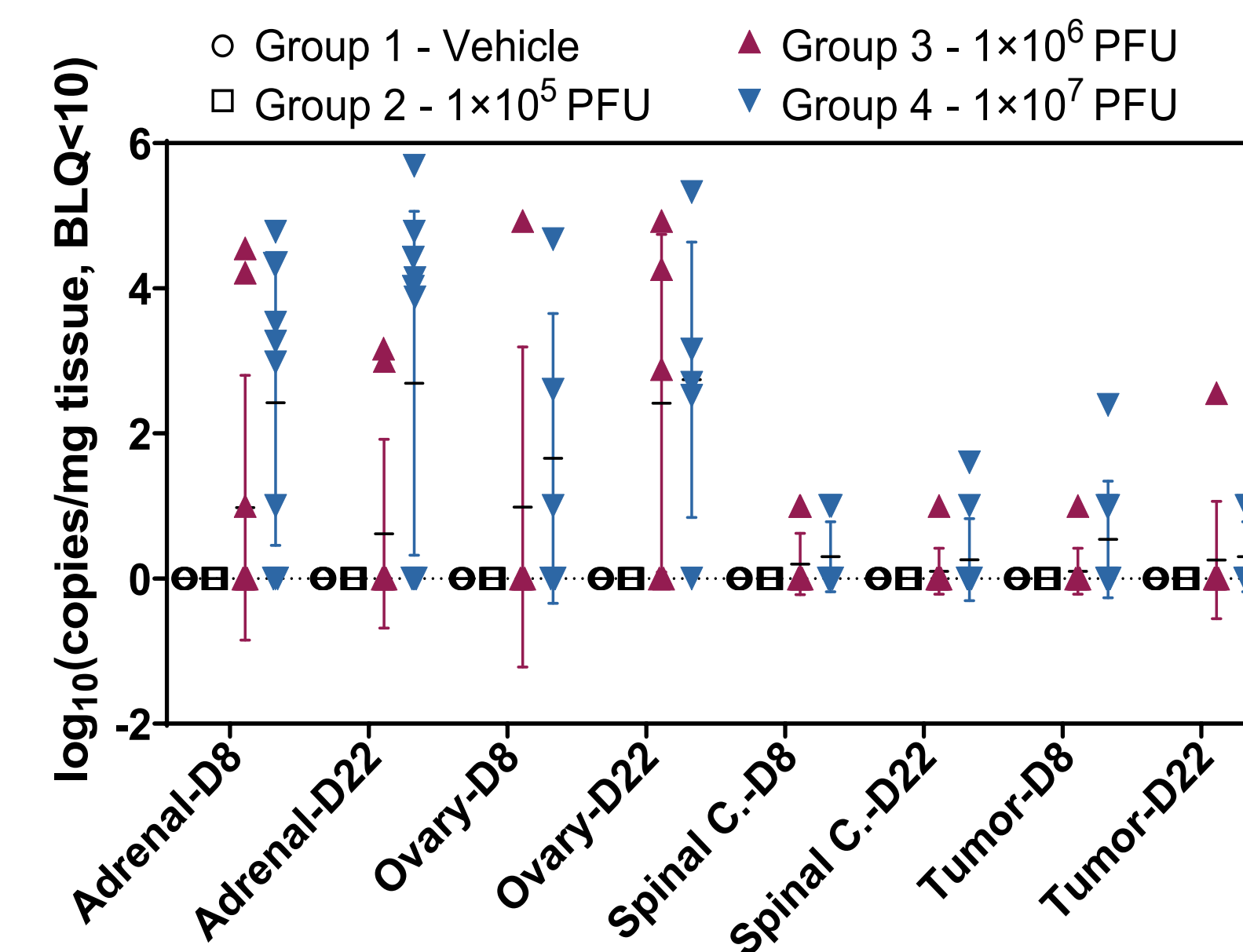
Dose-Dependent Suppression of Tumor Growth in Treated Hamster



Tumor growth kinetics and inhibition rate. The vehicle control group showed continuous and rapid tumor growth, while drug-treated groups exhibited reduced growth, especially at mid/high doses. (Left, N=8/14/20, mean $\pm$ SEM) On Day 22, inhibition reached 33%, 65%, and 81% in males and 27%, 72%, and 76% in females at ascending doses. (Right, N=8, mean $\pm$ SD)

3

Virus Mainly Detected in Adrenal Glands and Ovaries with No Apparent Shedding



Biodistribution. OV was detected in several tissues of G3 and G4 animals on D8 and D22, with a higher number of detections in G4 compared to G3 (N=10, mean $\pm$ SD).

No Ovs were detected in the feces or saliva, and low concentrations were detected in the injection site skin of one G4 male on D22, and in the urine of two G4 males on D8 and D22, respectively (not shown).

2

Virus Exposure Detected Mainly in the High-Dose Group with Subsequent Clearance Over Time, Time-Dependent Rise in Neutralizing Antibody Response

A High-Dose TK Exposure

Dose PFU/animal	Time (h)	Male		Female	
		Avg. Conc.	Exp / Total	Avg. Conc.	Exp / Total
1×10^7	0	BLQ	-	BLQ	-
	0.5	161	4/4	92	4/4
	2	28	3/4	28	1/4
	4	61	4/4	37	2/4
	8	21	1/4	BLQ	-
	24	BLQ	-	29	1/4
	72	BLQ	-	BLQ	-
	168	BLQ	-	BLQ	-

Note: BLQ: < 20 copies/ μ L, detected by validated qPCR method

Toxicokinetic. A: No virus was detected in the control or low-dose groups, only two males in the mid-dose group showed detectable levels at 0.5 h post-dose. In the high-dose group (1×10^7 PFU), the highest concentration was observed at 0.5 h post-dose, followed by a rapid monophasic decrease to non-quantifiable levels in both sexes. Because only a limited number of timepoints has virus detection, toxicokinetic parameter calculation could not be performed.

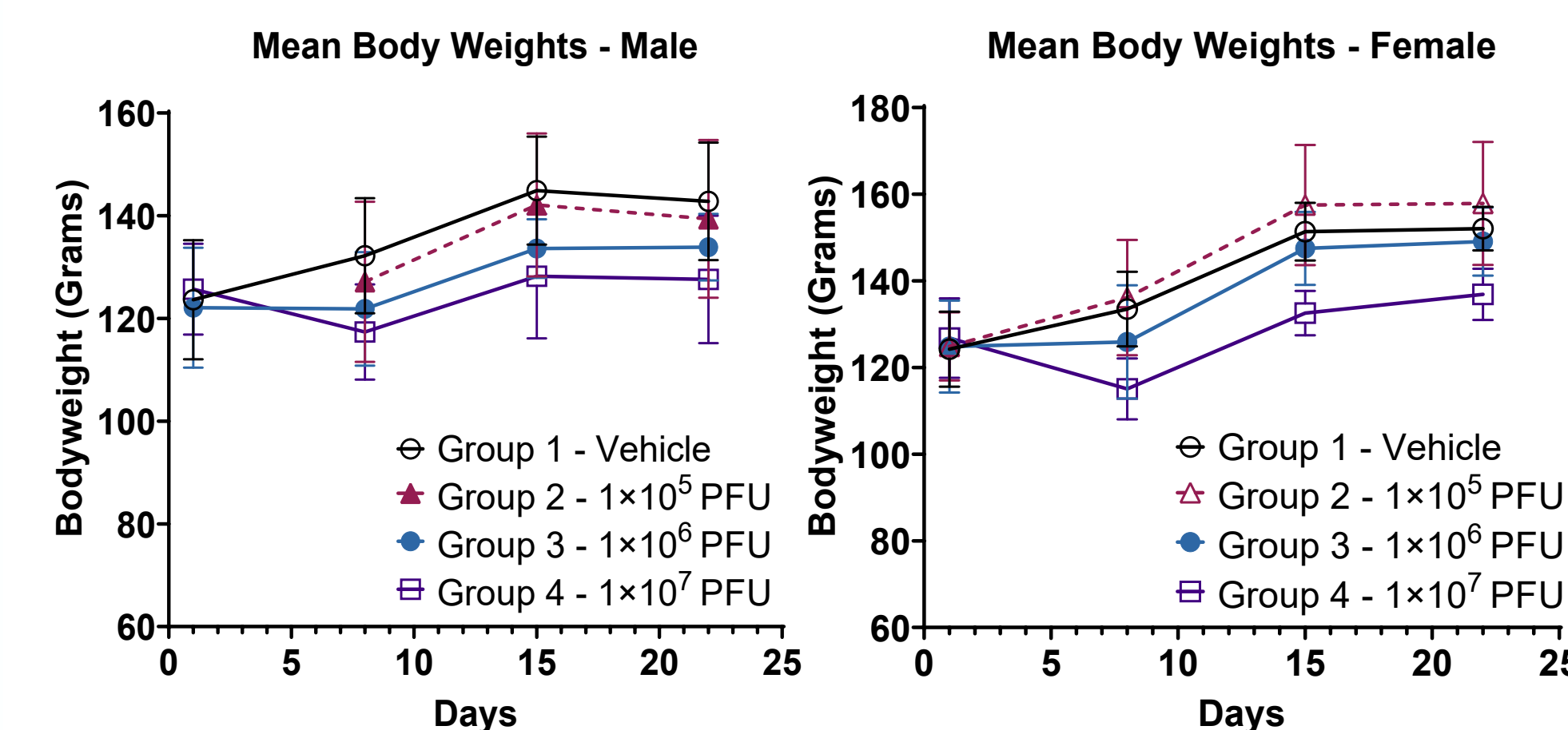
Neutralizing Antibodies. B: In all treated groups, the neutralizing antibody positivity rate increased over time post-administration, reaching 100% on Day 22.

B Neutralizing Antibody Positive Rate and Antibody Titers

Dose PFU/animal	Item	PreD	Day8	Day15	Day22	Total Positive Rate
Vehicle	Positive rate	3/8	0/8	0/8	0/8	3/24
	Ab Titers	$\leq 1:14$	$\leq 1:10$	$\leq 1:10$	$\leq 1:10$	
1×10^5	Positive rate	2/8	2/8	4/8	8/8	15/24
	Ab Titers	$\leq 1:11$	$\leq 1:16$	$\leq 1:23$	1:21-1:61	
1×10^6	Positive rate	0/8	3/8	7/8	8/8	18/24
	Ab Titers	$\leq 1:10$	$\leq 1:23$	$\leq 1:40$	1:29-1:173	
1×10^7	Positive rate	0/8	4/8	8/8	8/8	20/24
	Ab Titers	$\leq 1:10$	$\leq 1:62$	1:26-1:121	1:84-1:201	

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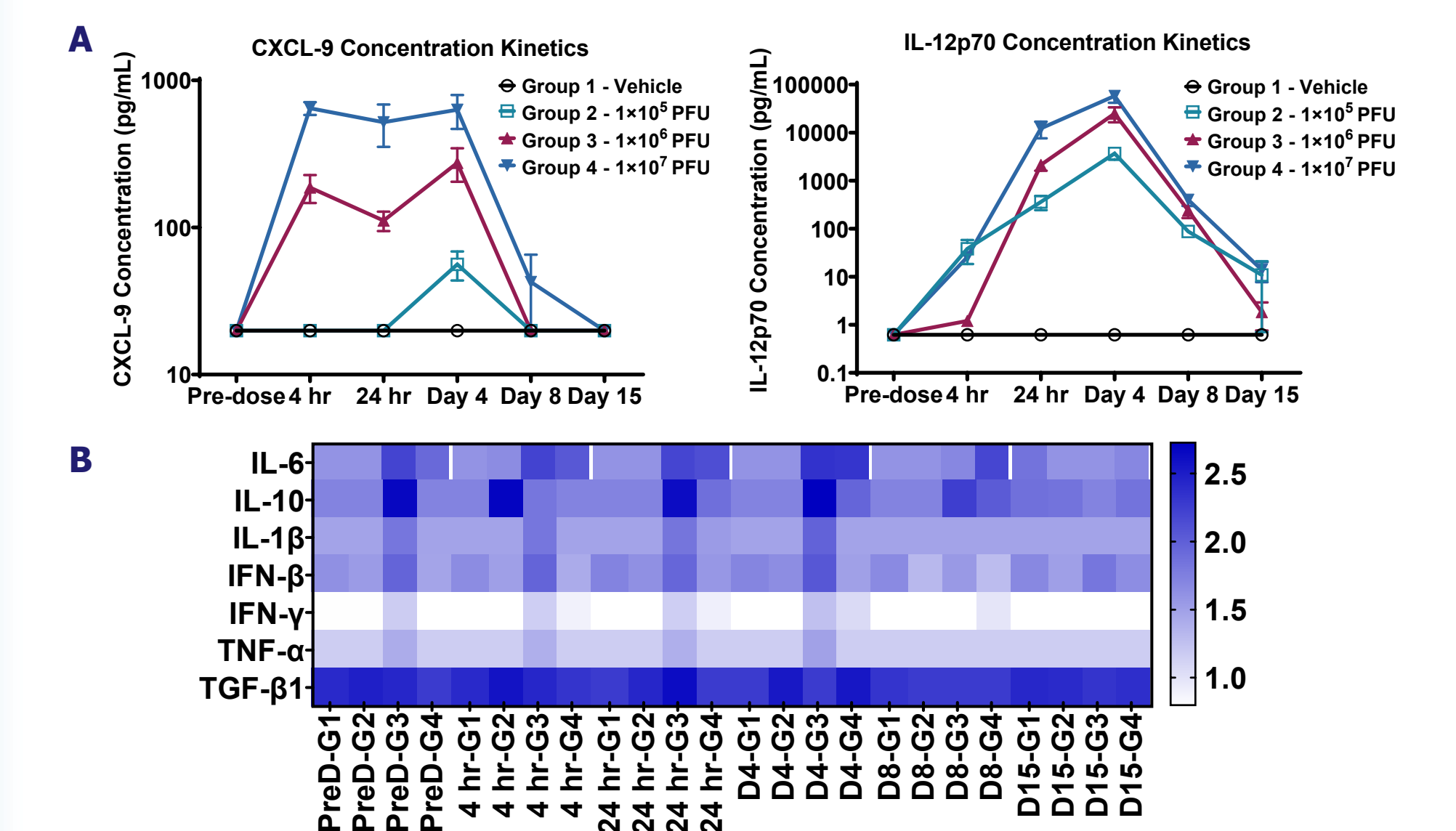
Body Weight Changes During the Study Period



Mean body weights. Test article-related decrease in body weights noted on Day 8 (8% in male and 6% in female given 1×10^6 PFU; 11% ($p < 0.01$) in male and 14% ($p < 0.001$) in female given 1×10^7 PFU). No significant difference noted thereafter. The food consumption is consistent with the weight loss. (D1: N=20, D8: N=14, D14/D22: N=8, mean $\pm$ SD)

5

Virus-mediated oncolysis, IL-12p70 immunity, and CXCL-9 recruitment establish a local anti-tumor loop without systemic cytokine toxicity



Cytokine response profile. A: Human CXCL-9 and IL-12p70 in a dose-dependent manner. B: Hamster IL-6, IL-10, IL-1 β , IFN- β , IFN- γ , TNF- α , and TGF- β 1 were detected in 1 to 8 animals across all groups, including the vehicle control. Detection occurred at pre-dose or low concentrations, with variable incidence and no dose-dependent pattern. (N=8, mean $\pm$ SEM)