

EG-70, A Novel Non-Viral Gene Therapy for Local Expression of Innate and Adaptive Immune Modulators for Treatment of Non-Muscle Invasive Bladder Cancer

enGene

Dauphinee SM*, Goulet ML*, Veilleux D, Louis KS, Lazure D, Stevenson S, Bilimoria D, Dupaul-Chicoine J, Sublemontier S, Amirkhani S, Fleet C, Bojic L, Vancutsem P, Tan S, Marquis JF, Lora JM*, Cheung AT*

*Authors contributed equally to this work
*Corresponding authors (acheung@engene.com)

Introduction

enGene has developed a novel non-viral gene therapy platform for delivering nucleic acids to mucosal tissues. Based on this proprietary platform, EG-70 is a genetic medicine designed to localize expression of immune modulators to the bladder mucosa for the treatment of Bacillus Calmette-Guérin (BCG)-unresponsive non-muscle invasive bladder cancer (NMIBC). The EG-70 drug product contains a non-integrative plasmid DNA drug substance packaged in dually-derivatized oligochitosan (DDX). The resultant nanoparticles are further coated with a PEG-b-poly-L-glutamic acid diblock copolymer. The plasmid DNA of EG-70 encodes the two subunits (p40 and p35) of human interleukin (IL)-12, expressed as a single-chain protein, in addition to two ribonucleic acid (RNA) products, eRNA11a and VA1, that activate the retinoic acid inducible gene I (RIG-I) pathway. Given that human IL-12 lacks biological activity in mice, a mouse surrogate (mEG-70) that expresses mouse IL-12 protein was used for preclinical pharmacology studies. The combination of IL-12 and RIG-I agonists was designed to locally activate pathways in the bladder for synergistic stimulation of anti-tumor immune responses without the risk of systemic toxicities.

Results

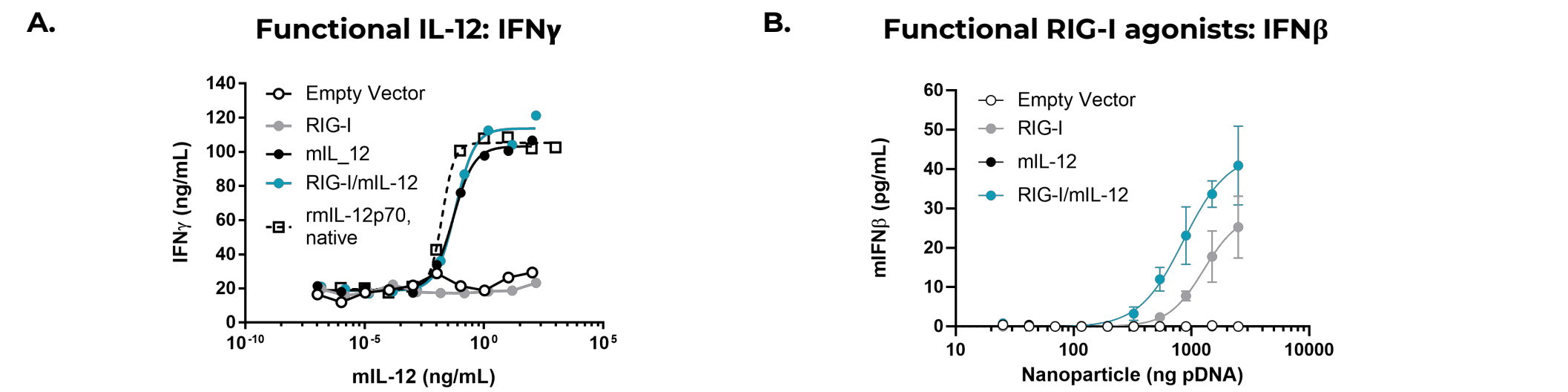


Figure 1. *In vitro* potency. (A) Mouse splenocytes (C57BL/6J mice) were stimulated with anti-mCD3 (0.2 μ g/mL) and anti-mCD28 (0.5 μ g/mL) $\pm$ IL-12 (supernatants from transfected cells) for 48 h and IFN γ was measured by immunoassay. (B) Mouse bladder cancer (MB49) cells were transfected with increasing doses of nanoparticles and supernatants were collected at 72 h post-transfection to quantify mouse IFN β .

Bladder epithelial cells produce functional IL-12 and RIG-I agonists transgene products

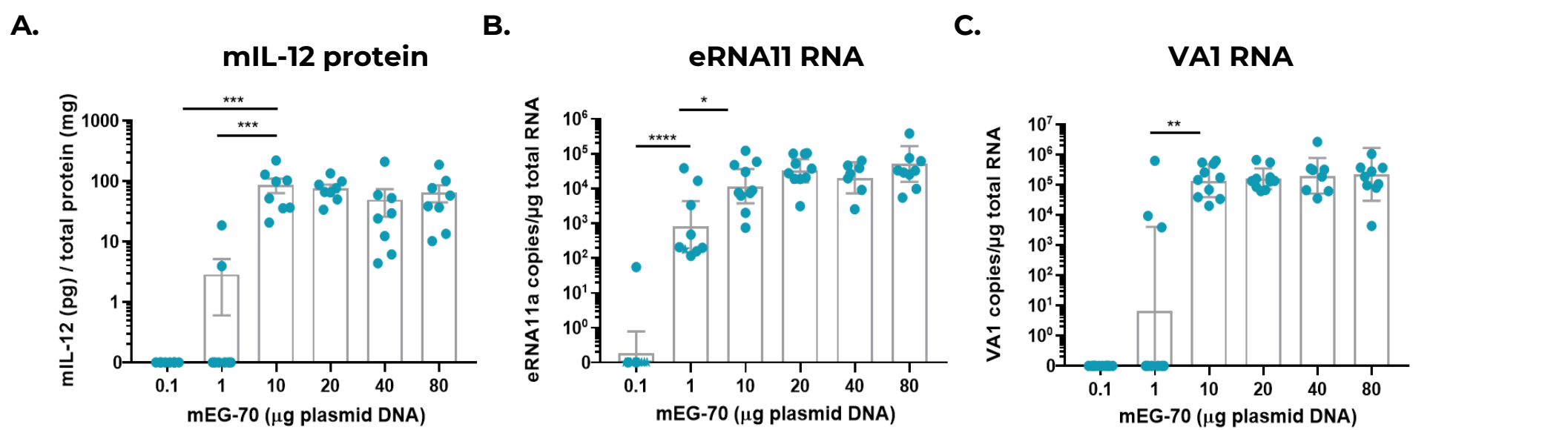


Figure 2. Expression of transgenes *in vivo*. Female C57BL/6J mice received a single intravesical instillation (IVI) of mEG-70 nanoparticles at increasing doses, as indicated for (A) tissue lysis for immunoassay of IL-12 protein; (B-C) RNA extraction and RT-qPCR of eRNA11a (B) or VA1 (C). Each data point represents expression in a single mouse bladder. n=8/group; data are presented with geometric mean $\pm$ 95% CI; *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001; Mann Whitney test. There was no mIL-12 protein detected in plasma of mEG-70-treated animals.

Transgene products show dose-dependent expression in mouse bladders

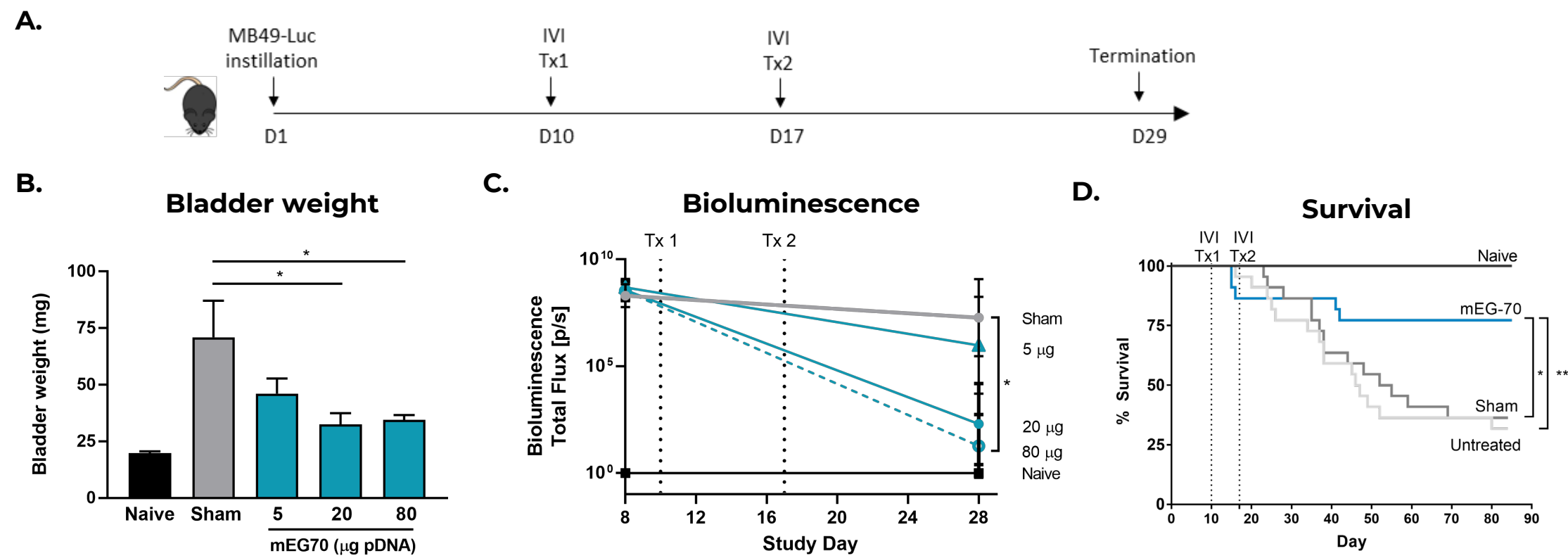


Figure 3. Efficacy of mEG-70. (A) An orthotopic model of bladder cancer was used to evaluate efficacy using two IVI of mEG-70 or 1% mannitol (sham) on Day 10, 17 (Tx1, Tx2); (B) Bladder weight was measured at endpoint for all groups (n=9-11/group, mean $\pm$ SEM); (C) Luminescence at Day 8 (randomization) and endpoint; *p<0.05; unpaired Student t test (D) Survival was monitored for 85 days (n=16-21/group; *p<0.05, **p<0.01 by log-rank (Mantel-Cox) test).

mEG-70 elicits profound dose-dependent therapeutic benefit in a mouse model of bladder cancer

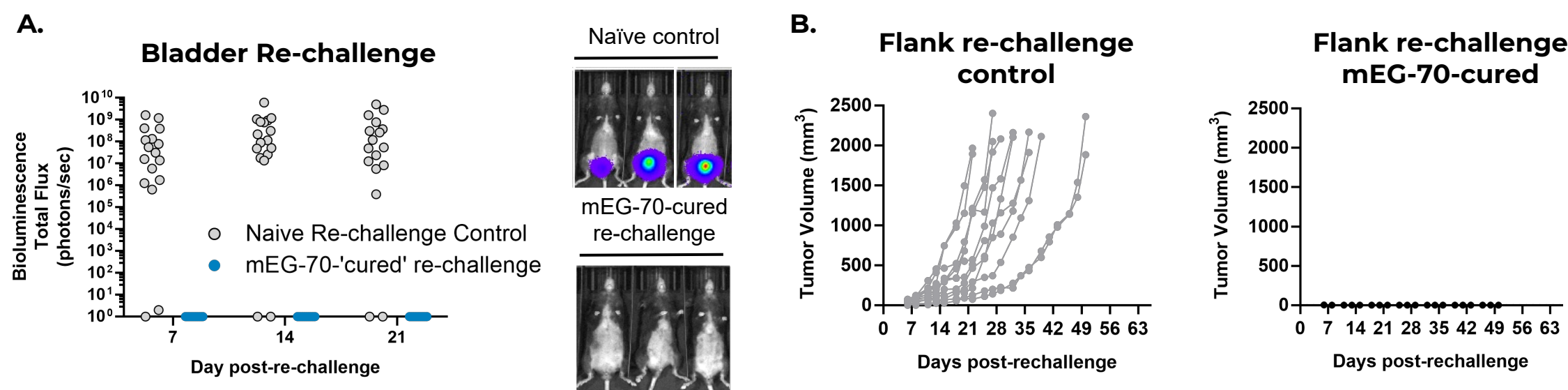


Figure 4. Systemic anti-tumor immunity. (A) mEG-70-cured mice were re-challenged by IVI of MB49-Luc cells and tumors were monitored by *in vivo* imaging; representative animals 13 days post re-challenge. (B) mEG-70-cured mice were re-challenged on the flank with MB49-Luc (n=12-15/group). Age-matched mice were challenged as controls.

mEG-70 treatment results in durable and systemic anti-tumor immunity

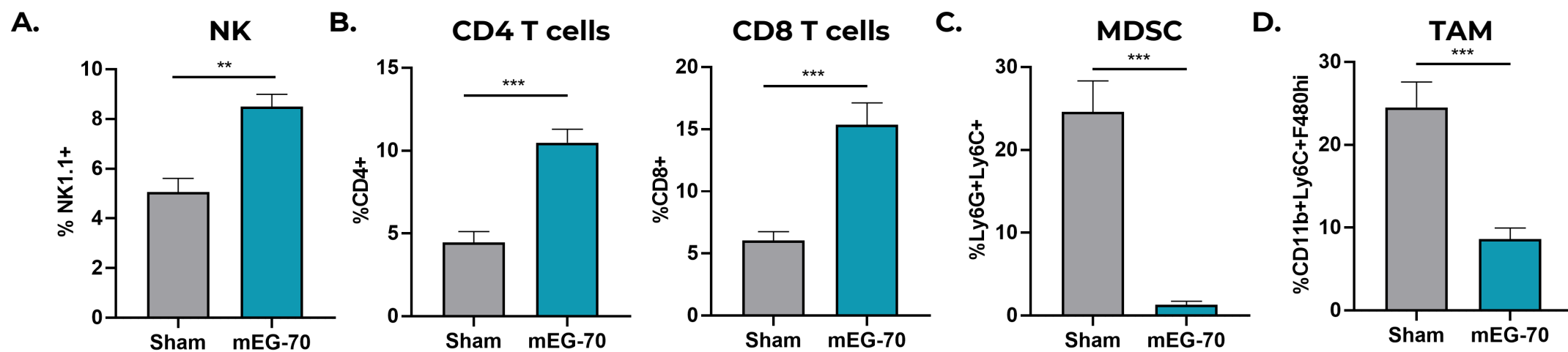


Figure 5. Immunophenotyping. Tumor bearing mice received IVI of mEG-70 or 1% mannitol (sham) on Day 10, 17. Bladders were harvested for flow cytometry analysis of (A) NK cells (NK1.1); (B) T cells (CD4+ or CD8+); (C) MDSC (Ly6G+Ly6C+CD11b+) and tumor-associated macrophages (TAM; CD11b+Ly6C+F4/80hi) (n=8/group; mean $\pm$ SEM. **p<0.01, ***p<0.001; Mann Whitney test.

mEG-70 elicits profound dose-dependent therapeutic benefit in a mouse model of bladder cancer

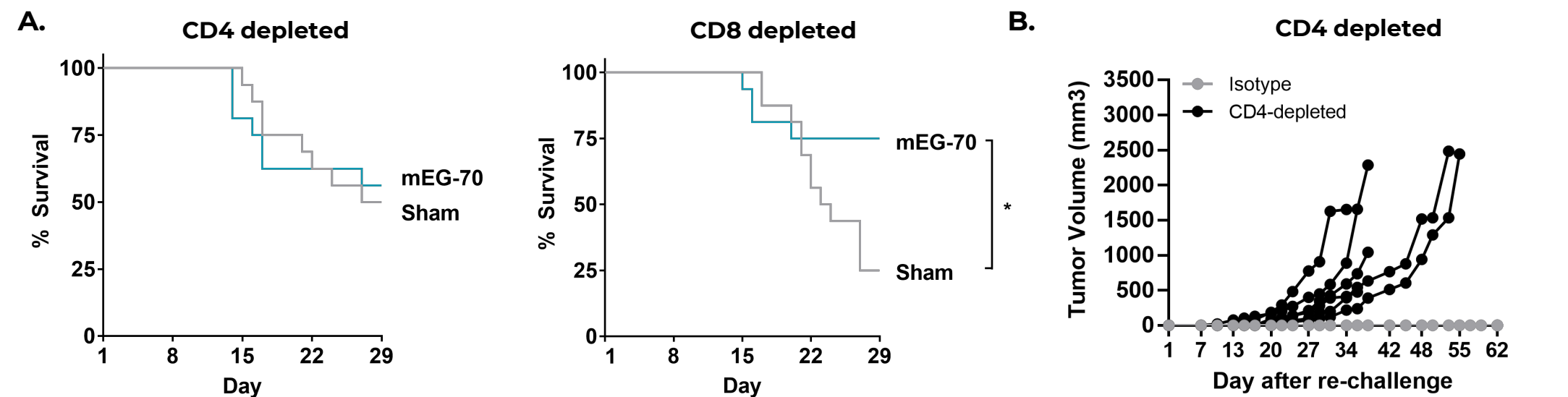


Figure 6. T cell depletion. Mice were injected intraperitoneally with anti-mCD4 or anti-mCD8 for 4 consecutive days to establish depletion prior to bladder instillation of MB49-Luc on Day 1. Mice received two IVI of mEG-70 or 1% mannitol (sham) on Day 10, 17. Antibody depletion of T cells was continued twice a week throughout the study. (A) Mice were monitored for survival for 29 days (n=16/group; *p<0.05 by log-rank (Mantel-Cox) test). (B) Non-depleted mEG-70-cured mice were re-challenged on the flank with MB49-Luc and tumors were monitored by measuring with a caliper and calculated using tumor volume = length x width²/2; (n=6/group).

CD4+ T cells are important for mEG-70-mediated local and systemic anti-tumor immunity

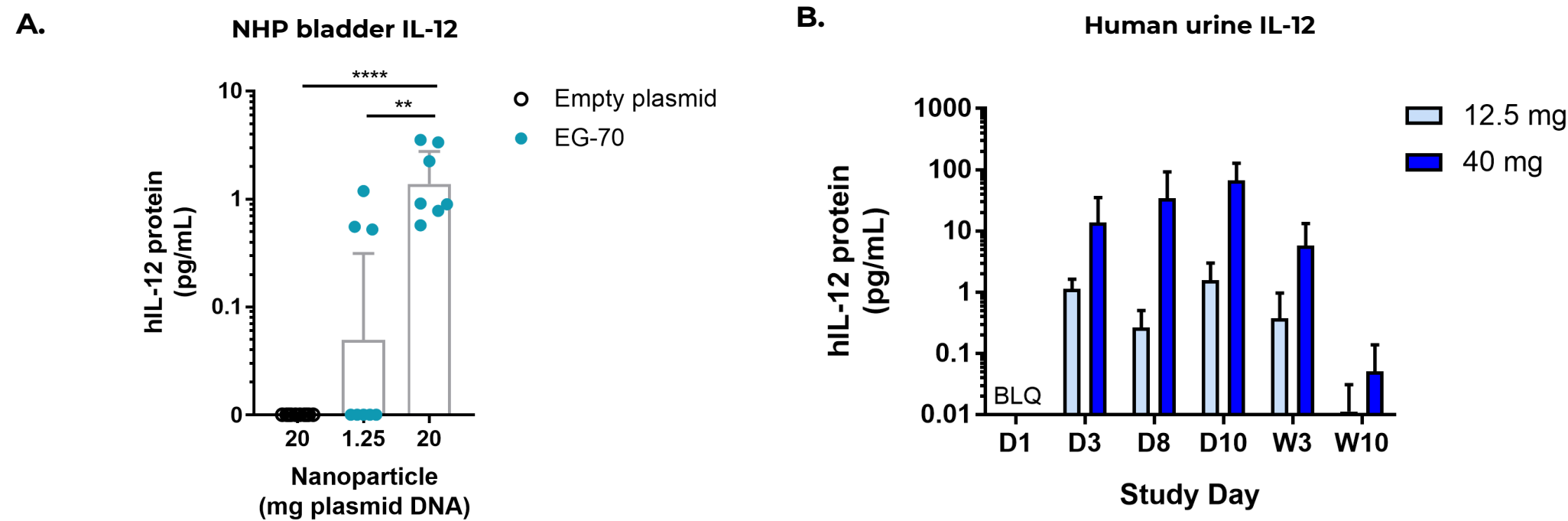


Figure 7. IL-12 expression in NHP bladders and human urine. (A) Cynomolgus monkeys received one administration of nanoparticles and bladder tissue was harvested at 48 h post-administration. Each NHP bladder was divided into 6-8 fragments and pulverized tissue was used to quantify hIL-12 protein by immunoassay (n=1 NHP/group), mean $\pm$ SEM for multiple fragments of an NHP bladder; **p<0.01, ***p<0.0001 by Mann-Whitney test. (B) Patients received two administrations of EG-70 on Day 1, 8 and urine was collected at indicated times for hIL-12 protein quantitation by immunoassay (n=3 patients/group, mean $\pm$ SD).

IL-12 protein is expressed in monkey bladders and human urine, demonstrating translatability

Conclusions

Intravesical instillation of mEG-70 results in robust transgene expression in the bladder mucosa.

mEG-70 elicits the intended immunological effects, which drives durable therapeutic benefit and systemic anti-tumor immunity in an orthotopic mouse model of bladder cancer.

EG-70 is well-tolerated in the preclinical setting with minimal and reversible toxicity.

A first-in-human Phase1/2 clinical trial (the LEGEND study, NTC04752722) is ongoing to evaluate safety and efficacy of intravesical administration of EG-70 in patients with BCG-unresponsive NMIBC.

Dose-dependent expression of IL-12 is observed in monkey bladders and human urine samples from LEGEND.