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# Assessing lentiviral vector multiple re-dosing schedules for improving and sustaining transgene expression

Chantelle Carpentieri<sup>1-3</sup>, Martin Donnelley<sup>1-3</sup>, Patricia Cmielewski<sup>1-3</sup>, Nathan Rout-Pitt<sup>1-3</sup>, Juliette Delhove<sup>1-3</sup>, David Parsons<sup>1-3</sup> and Nigel Farrow<sup>1-3</sup>

1. Robinson Research Institute, University of Adelaide, SA, 5001, Australia

2. Adelaide Medical School, University of Adelaide, SA, 5001, Australia

3. Department of Respiratory and Sleep Medicine, Women's and Children's Hospital, SA, 5006, Australia

## Introduction

- Lentiviral (LV) gene vectors are a promising option for treating cystic fibrosis airway disease by delivering a functional copy of the *CFTR* gene into airway epithelial cells.
- Our two-step lysophosphatidylcholine (LPC) conditioning and VSV-G LV vector delivery system successfully transduces airway epithelial cells in multiple animal models. However, achieving high levels of sustained gene expression remains challenging.
- To provide lifelong therapeutic gene expression, it may be beneficial to produce higher levels of initial gene expression through initial multi-dosing, and to be able to effectively repeat-dose if gene expression wanes over time.
- Previous studies have examined strategies for effectively re-administering LV vectors to the airways and some have been successful without loss of effectiveness.
- It is currently unclear whether repeat LV dosing can increase expression levels, and if so, which timing strategy is best.

## Aims

- To determine the optimal multi-dose strategy to increase initial levels of airway gene expression, compared to our standard single dose protocol.
- To examine whether repeat-dosing at a later time-point can sustain lung expression levels if gene expression wanes.

## Methods

- The lungs of normal C57Bl/6 female mice (n = 9-12/group) were conditioned with 10 µl of LPC, followed one hour later by 20 µl of VSV-G HIV-1 gene vector containing either the Fluc-F2A-eGFP bicistronic cassette driven by the EF1α promoter or the nuclear-localised *LacZ* gene driven by the MPSV promoter.
- For part 1 of the study, mice were randomly separated into six multi-dosing schedule groups;
  - A = 1 dose (Single dose control)
  - B = 2 doses 1 day apart (2 x 1d)
  - C = 3 doses 3 days apart (3 x 3d)
  - D = 2 doses 1 week apart (2 x 1w)
  - E = 3 doses 1 week apart (3 x 1w)
  - F = 5 doses 1 month apart (5 x 1m)
- For part 2 of the study, mice were randomly separated into four repeat-dosing groups;
  - Luc (Single dose control)
  - Luc + Luc
  - Luc + LacZ
  - LacZ + Luc
- Bioluminescent imaging (BLI; IVIS Lumina XRMS) was performed at various time-points post LV vector instillation to assess *Luciferase (Luc)* gene expression over time.
- Blood samples have been collected pre-dosing and at all imaging timepoints. These will be analysed to assess immune responses.
- Lung tissue samples have been harvested and will be analysed to assess GFP and LacZ expression.

## Results

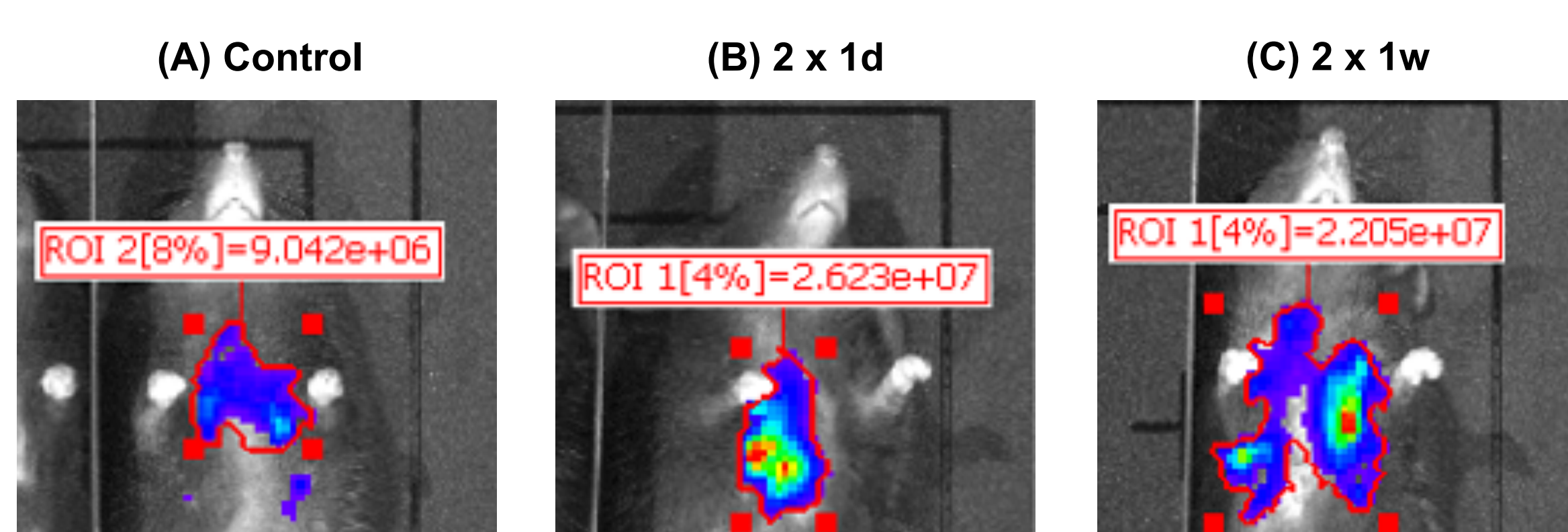


Figure 1: Example of *Luc* gene expression (flux bioluminescence) images from mice in three different dosing groups. (A) Control, (B) 2 x 1d apart (at 1 week BLI imaging timepoint), and (C) 2 x 1w apart (at 1 month BLI imaging time-point). Radiance scale p/sec/cm<sup>2</sup>/sr.

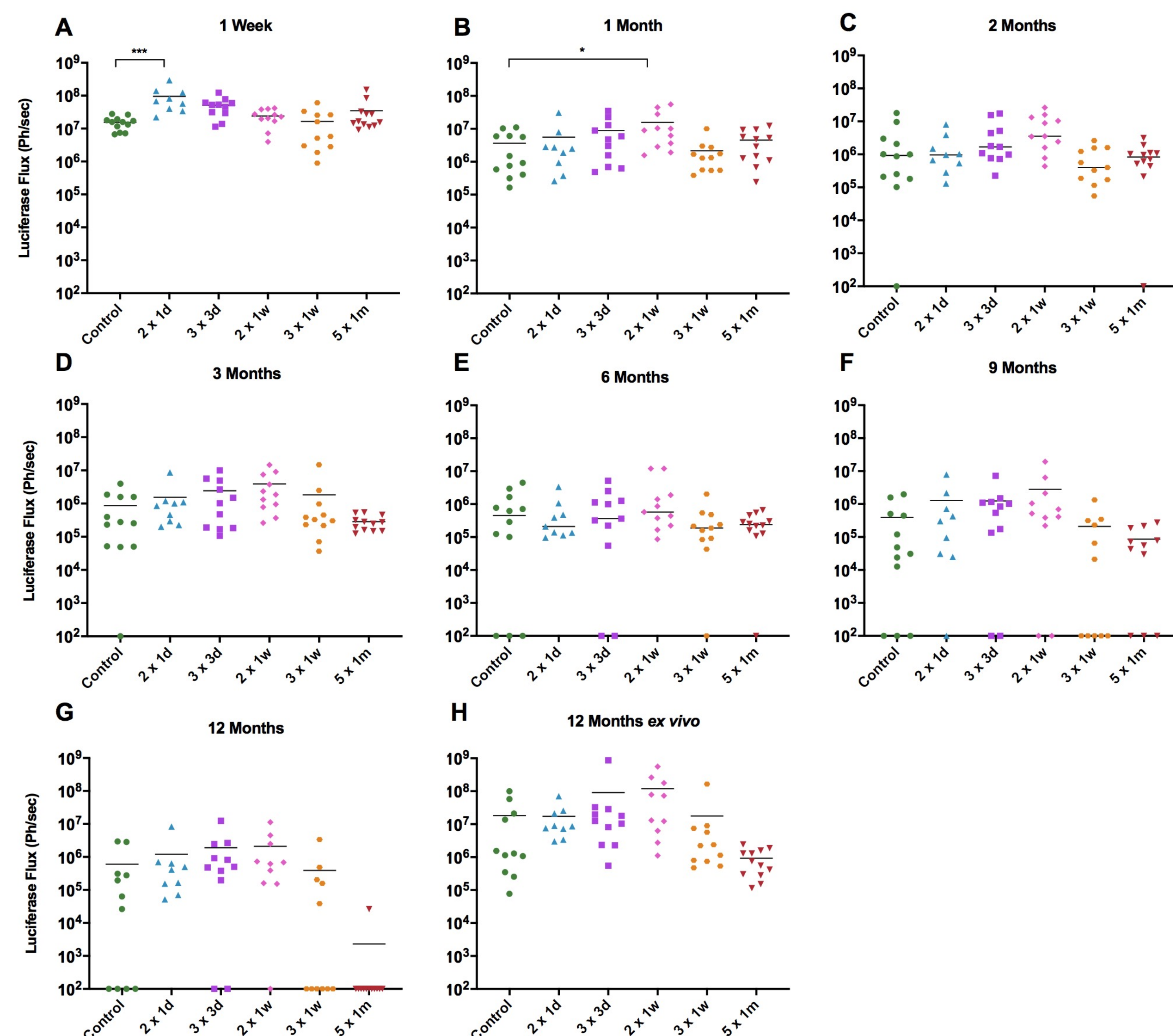


Figure 2: Part 1 results. *Luc* expression (flux bioluminescence) in the lung airways of mice following multi-doses of a LV gene vector. (A) Transgene expression at 1 week was higher in the 2 x 1d group compared to the control group. (B) At 1 month, transgene expression was higher in the 2 x 1w group compared to the control group. (C-G) At the later time-points there was no significant difference in transgene expression between any multi-dosing groups compared to the control group. (H) *Ex vivo* imaging at 12 months showed that there was no significant difference in transgene expression compared to the control group. \*\*\* p<0.0001, \* p<0.01, one-way ANOVA vs control, (n = 9-11 per group).

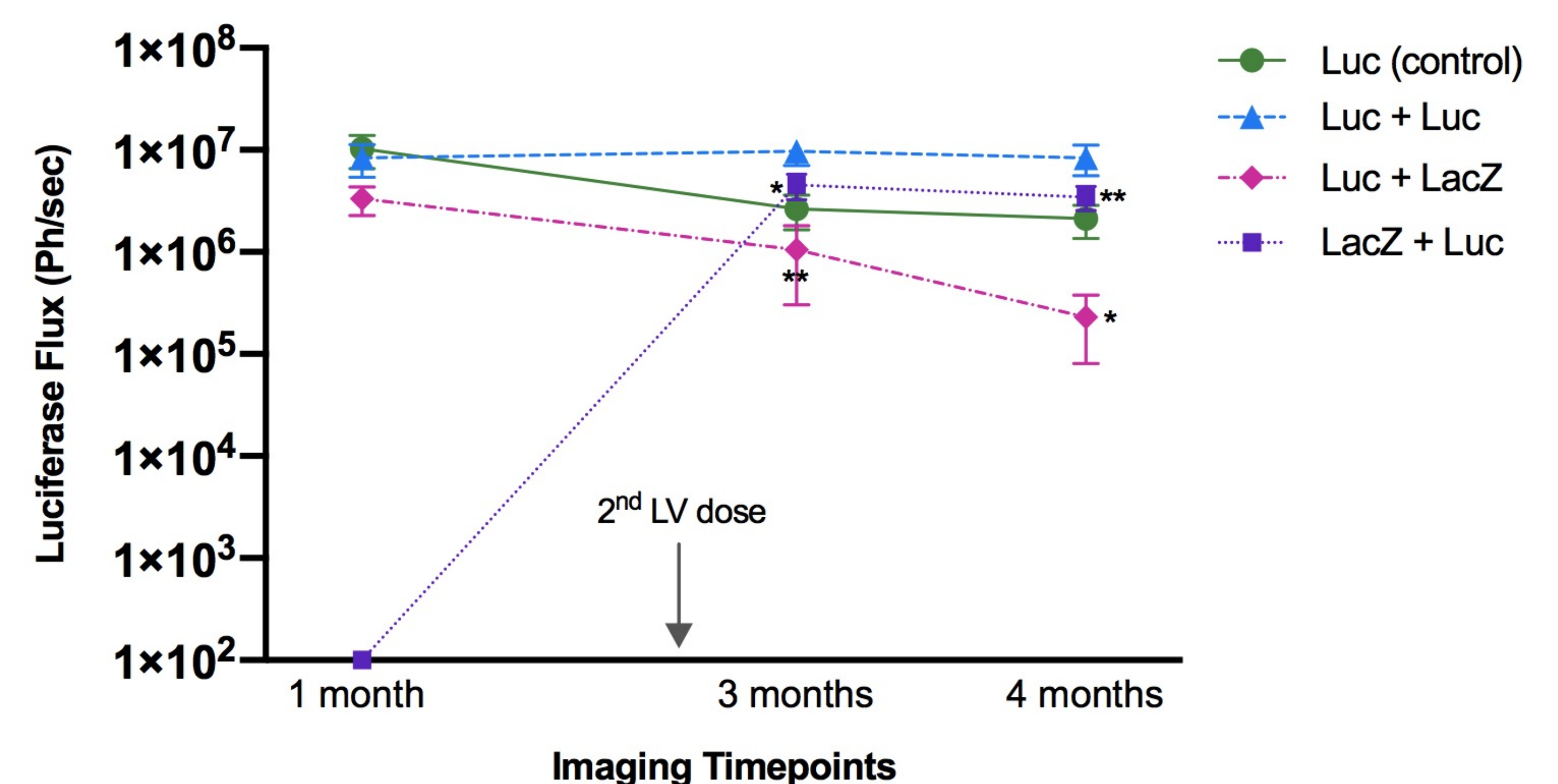


Figure 3: Part 2 results. *Luc* gene expression (flux bioluminescence) in the lung airways of mice following a repeat-dose of a LV vector containing either the same or a different transgene. *Luc* expression was significantly reduced in the Luc + LacZ group at 3 and 4 months, compared to the initial *Luc* dose. This was also seen in the LacZ + Luc group, however, the initial dose contained the *LacZ* transgene which has no luminescent properties and therefore cannot be detected by BLI. While there was no significant difference in the level of *Luc* expression, the Luc + Luc group produced and maintained higher levels of *Luc* expression compared to the control group. Mean with SEM, \* p<0.01, \*\* p<0.001, one way ANOVA, (n = 12 per group).

## Conclusions

- The results suggest that a VSV-G pseudotyped LV gene vector can be successfully readministered to the lung regardless of timing.
- Compared to our standard single dose delivery, there was significantly higher gene expression levels seen after 1 week following 2 doses 1 day apart, and at 1 month following 2 doses 1 week apart. After two months, there was no significant differences in flux between any of the multi-dosing schedules.
- Repeat-dosing with the same transgene is feasible and can maintain levels of transgene expression, while a repeat-dose with a different transgene resulted in a significant decrease in expression.



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