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

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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 VSV-G LV vector successfully transduces airway epithelial cells in multiple animal models, but 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 repeat-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.

Aim

- To determine the optimal repeat-dose strategy for producing robust and lasting gene expression levels.

Methods

- The lungs of normal C57Bl/6 female mice (n = 12/group) were conditioned with 10 µl of lysophosphatidylcholine (LPC), followed one hour later by 20 µl of VSV-G HIV-1 gene vector containing the Fluc-F2A-eGFP bicistronic cassette driven by the EF1α promoter.
- Mice were randomly separated into six dosing schedule groups;
  - 1 dose (Control)
  - 2 doses 1 day apart (2 x 1d)
  - 3 doses 3 days apart (3 x 3d)
  - 2 doses 1 week apart (2 x 1w)
  - 3 doses 1 week apart (3 x 1w)
  - 5 doses 1 month apart (5 x 1m)
- Bioluminescent imaging (BLI; Xenogen, IVIS) was performed at 1 week, 1, 2, 3, 6 and 9 months 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.
- BLI will continue until 12-months post-dosing, when tissues will be excised and analysed to assess GFP 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 timepoint).

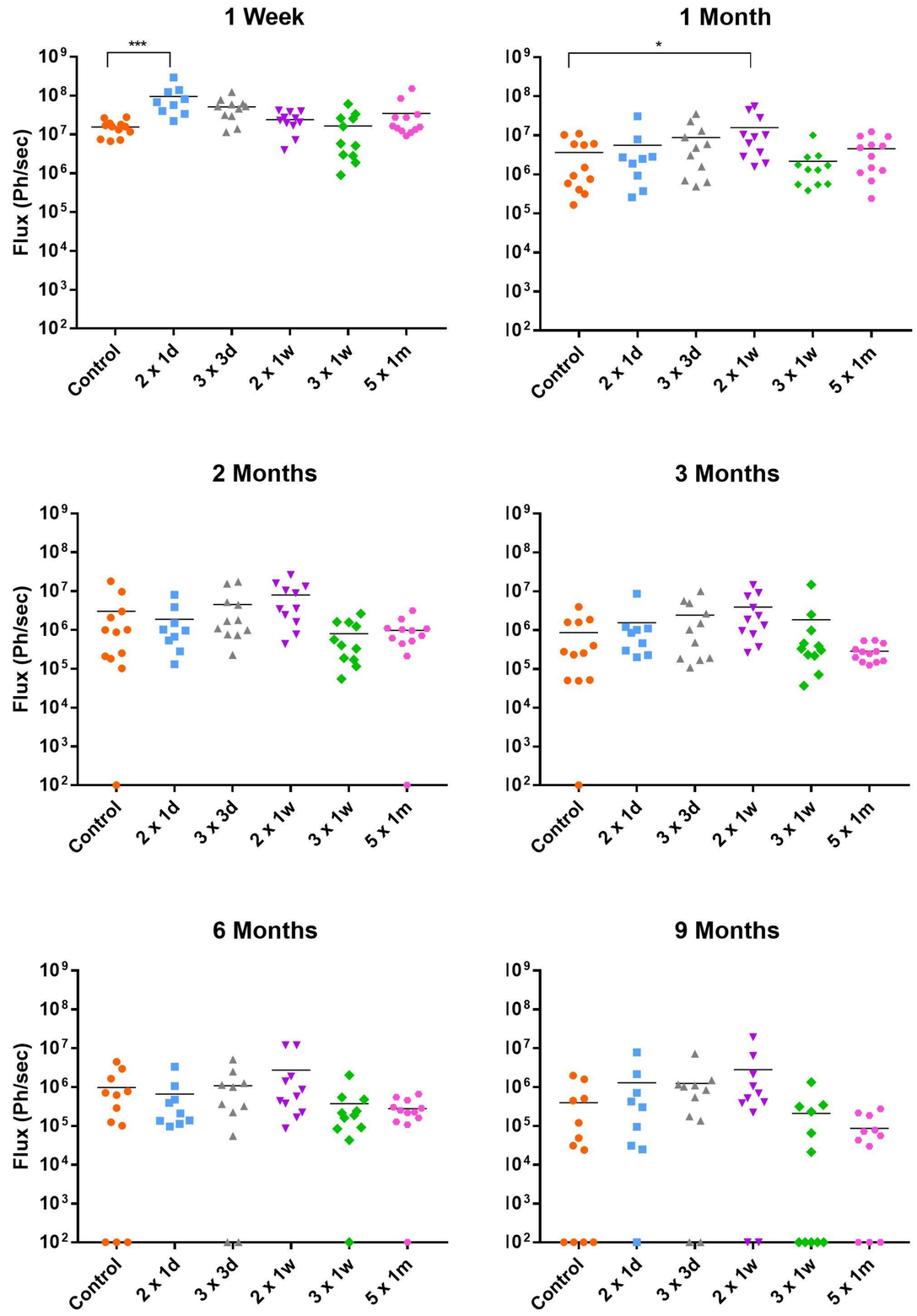

Figure 2: *Luc* gene expression (flux bioluminescence) in the lung airways of mice following repeat administration of LV gene vector. **(A)** Transgene expression at 1 week was higher in the 2 x 1d group compared to the control group. There was no significant difference between the remaining dosing schedules and the control. **(B)** At 1 month, transgene expression was higher in the 2 x 1w group compared to the control group. There was also no significant difference between the remaining dosing schedules and the control group. **(C-F)** At the later time-points there was no significant difference in transgene expression between any dosing schedule and the control group. *** p<0.0001, * p<0.02, one-way ANOVA vs control.

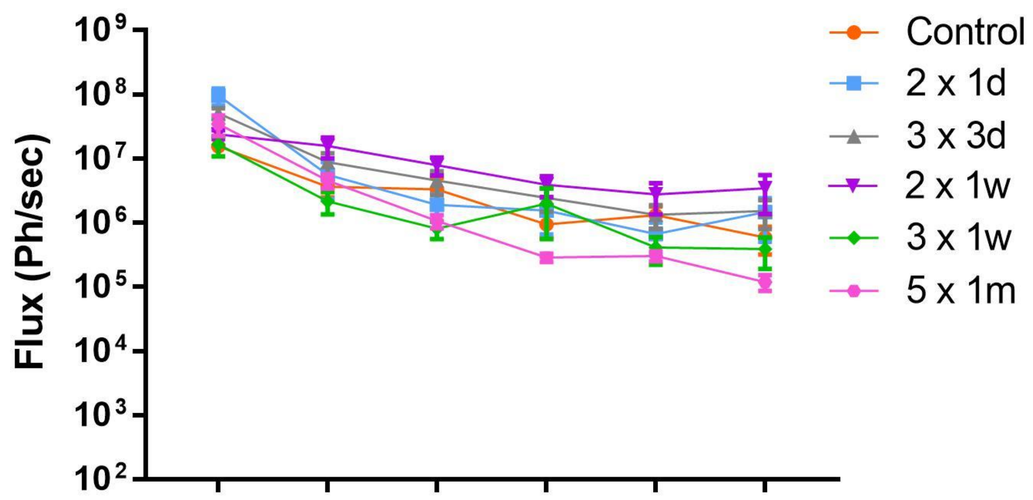

Figure 3: Average *Luc* gene expression (flux bioluminescence) in the lungs of mice over a 9 month period.

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 only 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 dosing schedules.

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