REPORTER GENE EXPRESSION FOLLOWING REPEAT ADMINISTRATION OF A HIV-1 LENTIVIRAL VECTOR IN MOUSE AIRWAYS

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BACKGROUND: Repeat dosing of a gene vector may be necessary to ensure long term correction for cystic fibrosis airway disease. Using luciferase (Luc) bioluminescence imaging, we have examined the persistence of gene expression in individual animals over time to determine if repeat dosing with a VSV-G pseudotyped lentiviral (LV) gene vector maintained gene expression in the airways of normal mice.

METHODS: Three groups of normal C57Bl/6 female mice (n=8/group) received a single VSV-G pseudotyped LV gene vector delivered intranasally (4µl of 0.3% lysophosphatidylcholine) followed 1 hour later by a 20µl bolus of the LV vector containing the Luc gene. Four weeks later, one group of mice was re-dosed with a different reporter-gene (LacZ), while another group was re-dosed with the same Luc gene. Luciferase bioluminescence (Fig.1a.) was measured 1, 4, 5, 8 and 12 weeks later (IVIS, Xenogen), 10 minutes after an intranasal delivery of the substrate D-luciferin (50µl, 15mg/ml). After humane killing all mice were analyzed for LacZ gene transduction via X-gal staining of histological sections (Fig. 1b.) and sera were analyzed via ELISA for circulating antibodies.

RESULTS: Nasal bioluminescence was the same across all 3 groups at 1-5 weeks post initial LV instillation (Fig. 2., n.s., ANOVA). Those mice that received a different transgene at re-dose (i.e. LacZ) displayed similar gene expression compared to the single dose group at both later time points. However at 8 and 12 weeks (Fig. 3., i.e. 2 months after the 2nd dose), mice that received two doses of Luc showed significantly less gene expression compared those given a single dose of Luc ($p<0.001$, ANOVA).

LacZ gene transduction was only detected in those mice that received the different transgene LacZ as the second dose (Fig. 4., $p<0.05$, ANOVA).

Circulating neutralising antibodies to both the transgene and the LV vector envelope (Fig. 5.) were detected in the sera, regardless of the number of doses given.

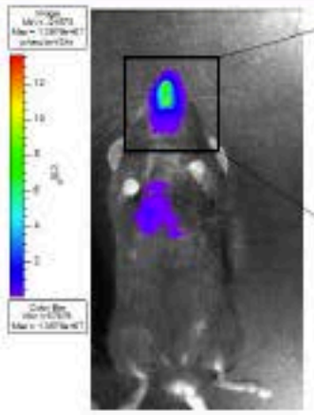

Fig. 1a. Bioluminescence measured 10 minutes after D-Luciferin substrate.
Insert: Nasal bioluminescence calculated as Flux (Photons/sec).

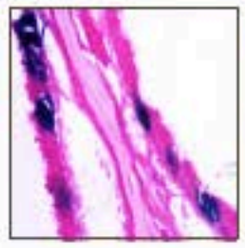

Fig. 1b. LacZ transduced nasal ciliated respiratory epithelium (blue cells) after X-gal staining. (H&E, x100 magnification)

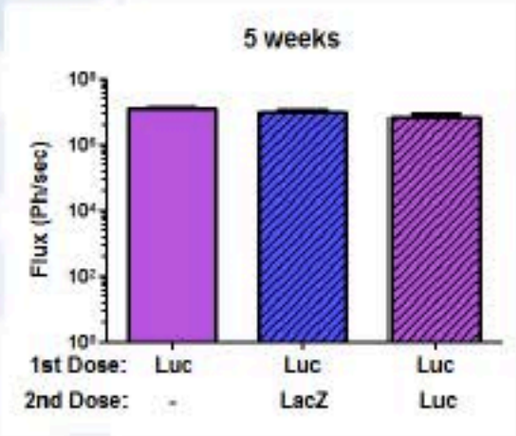

Fig. 2. No significant difference in nasal bioluminescence between all three groups at 5 weeks (n.s., ANOVA).

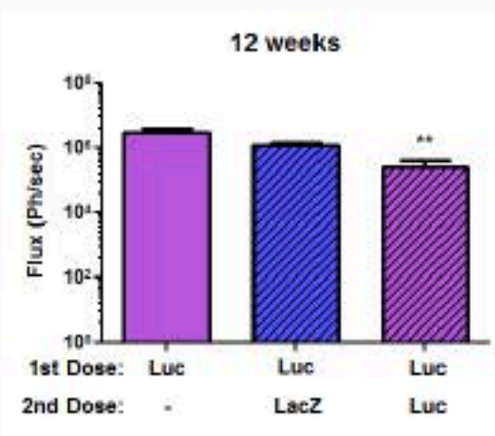

Fig. 3. Significant reduction in nasal bioluminescence in those mice that received two doses of Luc (**$p<0.001$, ANOVA).

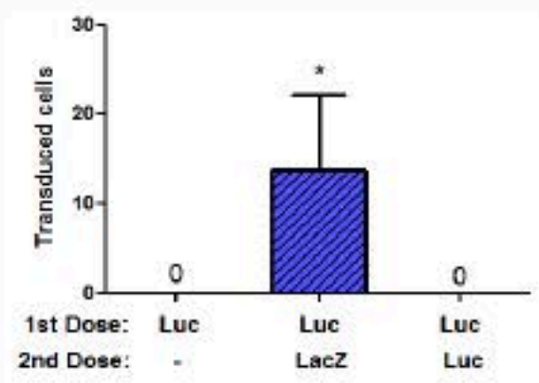

Fig. 4. Mice that received LacZ as the second dose displayed significant nasal LacZ gene expression at week 12 (*$p<0.05$, ANOVA, Range: 0-66 blue transduced cells, 2/8 = zero counts).

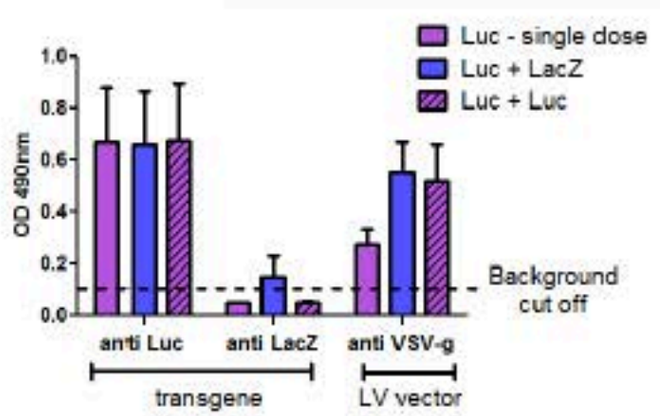

Fig. 5. Presence of circulating neutralising antibodies to the transgene and the LV vector envelope, in sera at week 12.

CONCLUSION: These results indicate that re-administration of our LV vector is possible. However re-administration of the same LV vector transgene after 4 weeks can reduce subsequent gene expression. This effect is likely to be primarily due to a cell-mediated immune response directed against the specific transgene.

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