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Enhancing lentiviral vector titre by limiting auto-transduction

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Introduction

- Lentiviral vector (LVV) is the most used tool for the delivery of genetic modification in cell therapies.
- With growing interest in the development of LVV-based *in vivo*-administered cell therapies, high-titre LVV production is of increasing importance.
- However, during LVV manufacturing, a fraction of LVV is lost to a process termed auto-transduction – the terminal re-entry of newly-produced LVV into the production cell line (**Figure 1**).
- To maximise LVV titres, it would thus be optimal to limit losses due to auto-transduction.
- During upstream LVV process optimisation, we identified an Additive X that boosts LVV production by limiting auto-transduction

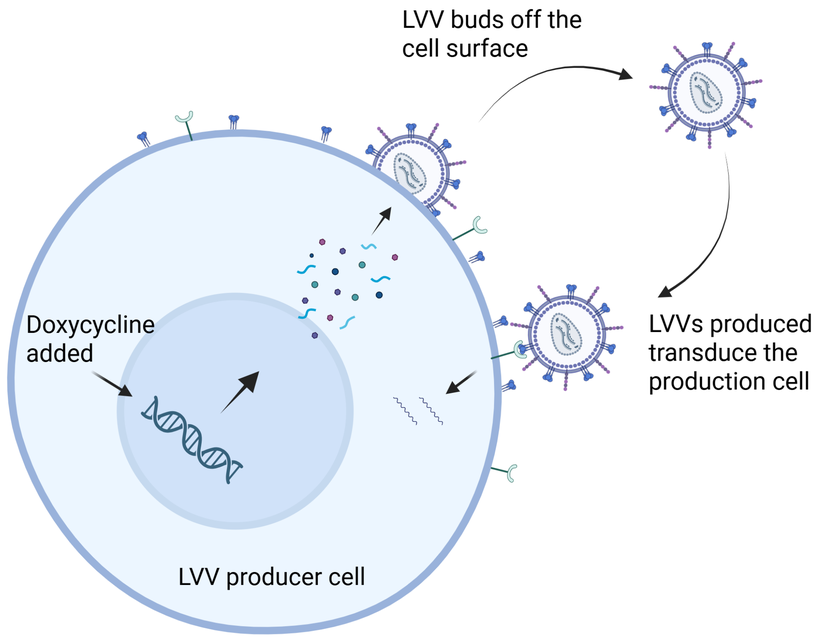

Figure 1: LVV titre limited by unintended auto-transduction of XOFLX producer cells. Schematic of auto-transduction.

Additive X increases infectious titre

- XOFLX Producer cells were induced by doxycycline, and LVV harvested 72 hours post-induction. Infectious titre was measured by both flow cytometry and dd-PCR.
- We have found that supplementing XOFLX producer cells with the Additive X increased infectious titre by 4.2-fold (**Figure 2**).
- For longer production processes there is potentially greater risk of loss of titre due to auto-transduction. We expect such processes would greatly benefit use of the Additive X.

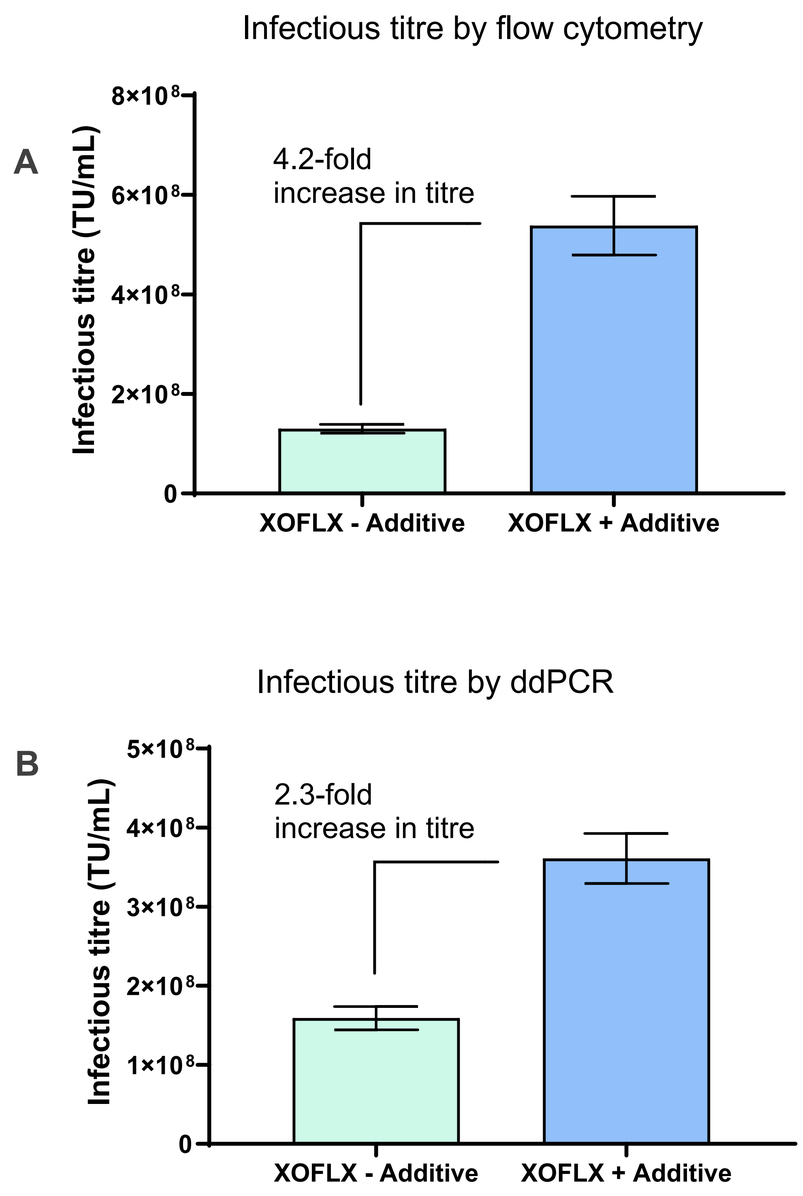

Figure 2. LVV Production with XOFLX EGFP producer cell line. (**A**) Infectious titre of LVV supernatant measured by flow cytometry. (**B**) Infectious titre of LVV supernatant measured by dd-PCR. Error bars indicate standard deviation (n=3).

Increase in LV titres correlates with reduction in auto-transduction

- In production cells, copy numbers of integrated Psi packaging signal were measured by dd-PCR.
- The addition of the Additive X reduced the integrated Psi copy numbers in the production cells by 50% (**Figure 3**), indicating a lower level of LVV auto-transduction.
- By reducing the auto-transduction events in the production culture, we can significantly improve the LVV yield.

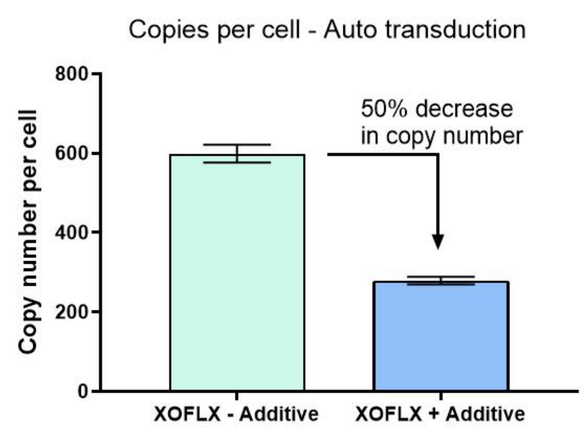

Figure 3. The Additive X limits auto-transduction during LVV production. Integrated vector copies in XOFLX producer cells. Error bars indicate standard deviation (n=3)

Additive X inhibits transduction

- We directly transduced suspension baseline cells in the absence or presence of the Additive X with the same concentration of LVV encoding EGFP for each condition.
- Integrated vector copies were measured by dd-PCR targeting Psi packaging element.
- Copy number in transduced cells with the Additive X was halved indicating inhibition of transduction (**Figure 4**).

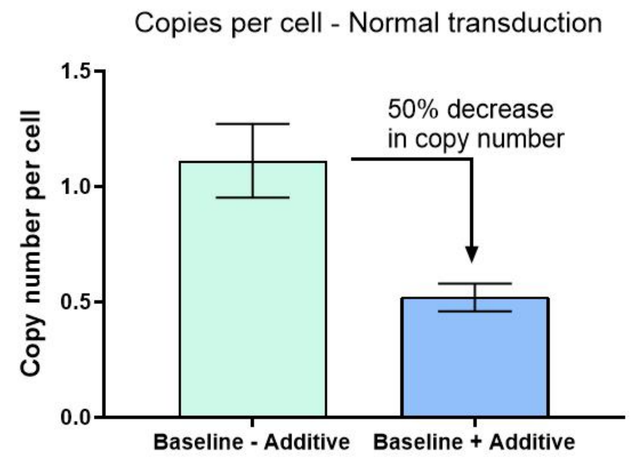

Figure 4. Effect of the Additive X on transduction. Integrated vector copies in transduced suspension cells. Error bars indicate standard deviation (n=3)

Optimisation of timings & dilutions

- We found that addition of the Additive X 48 hours post-seeding yielded higher titres compared to addition on the day of induction (**Figure 5**).
- Higher dilutions of the Additive X can be used regardless of the timing of the addition.

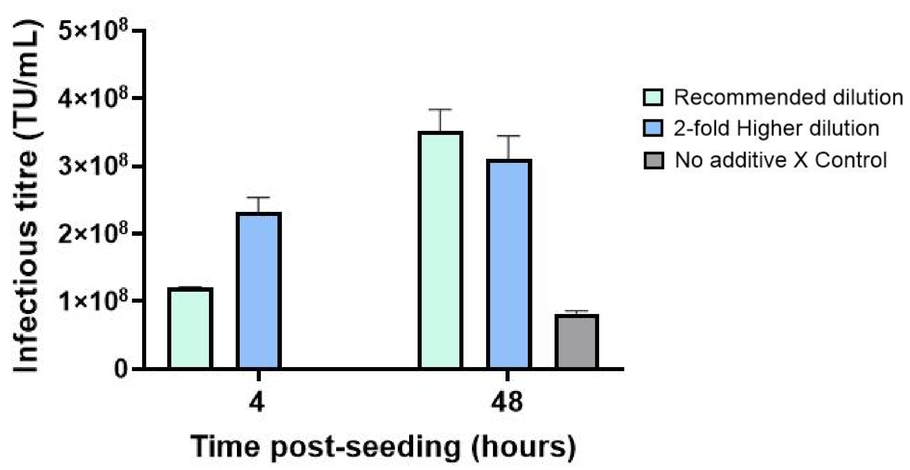

Figure 5. Assessment of LVV productivity with concentration and timing of the Additive X. Infectious titre measured by ddPCR. Error bars indicate standard deviation (n=3).

Conclusions

- We have identified an Additive X that limits auto-transduction of LVV back into the production cell line.
- Use of Additive X during LVV production boosts infectious titre by 4.2 fold.
- 50% reduction in vector copies observed in XOFLX producer cells with addition of the Additive X indicating reduced number of re-integration events.
- Comparable reduction in copy number was observed in transduced baseline cells supplemented with the Additive X. This confirmed that the Additive X acts by inhibiting auto-transduction.
- Recent optimisation experiments showed that addition of Additive X at 48 hours post-seeding was more beneficial than addition at induction.
- Use of Additive X would especially benefit continuous production processes where loss of titres due to auto-transduction could be an increased risk. Therefore, further investigation and optimisation into the use of the Additive X is needed for optimal use in the continuous production workflow.