Silencing cargo gene expression in lentiviral vector and AAV production to improve vector yield and purity

Qian Liu[1], Sherin Parokkaran Johny[1], Matthew Tridgett, Mohine Alam, Fran Bennett, Siobhan Clerkin, Michal Fleszar, Meenakshi Raghunath[1], Maria Patricio, Michael White, Weiheng Su[2], Vaughan Leydon[1]

[1] Minaris Advanced Therapies, London, UK, [2] Independent Consultant, Oxford, UK.

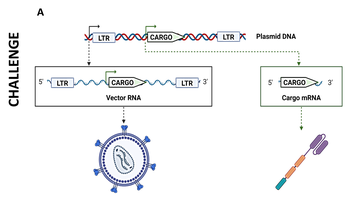
Minaris Advanced Therapies

Poster

1. Introduction

Lentiviral vectors (LVVs) and adeno-associated viral vectors (AAVs) are widely used as delivery platforms for genetic modification in both *ex vivo* cell therapies and *in vivo* gene therapies. However, their manufacturing can be complicated by the expression of certain therapeutic genes, particularly when these genes are cytotoxic. In such cases, cell death during production can generate byproducts that increase the burden on downstream processing.
These challenges are difficult to avoid because cargo gene expression is typically high, driven by the large quantities of transfer plasmid used during manufacturing. In addition, strong viral promoters positioned upstream of the cargo gene can unintentionally amplify expression within the production system.
As a result, there is a need to develop strategies to suppress cargo gene expression during production, enabling reliable manufacturing of viral vectors that carry potent but cytotoxic therapeutic genes.

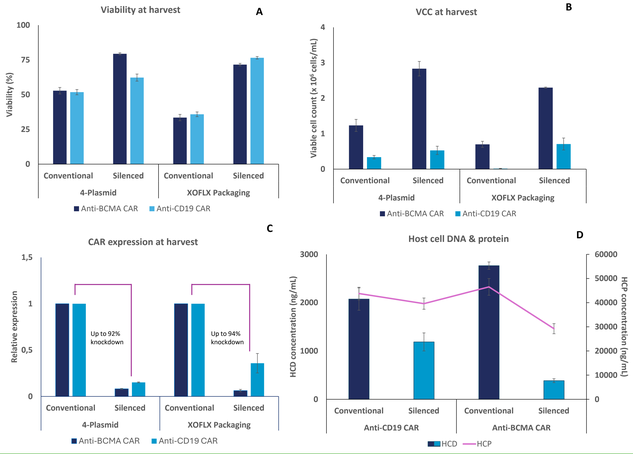
2. Mechanism of LVV cargo gene silencing



- LVV transfer plasmids generate vector RNA and cargo gene mRNA.
- Because the cargo gene mRNA sequence is entirely encoded within the vector RNA, standard silencing methods would limit LVV production.
- We have engineered a distinction between the two RNA species, enabling the use of shRNA silencing.
- Use of an UTR-based target site facilitates universal targeting by shRNA regardless of the cargo gene, thus enabling easy customisation.
- For optimal production, we also silence the antiviral gene *PKR*.

***Figure 1**: (A) Challenge associated with silencing LVV cargo gene, (B) Schematic of cargo-gene silencing system.*

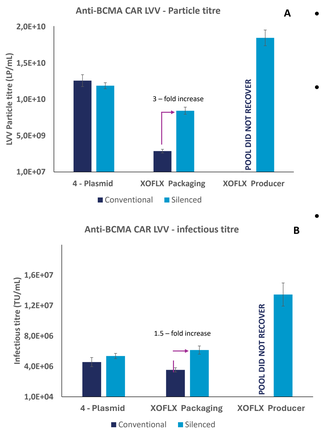
3. LVV Cargo gene knockdown improves production cell health & reduces DSP burden



- Silencing the cargo gene during LVV production improved the health profile of production cells in both 4-plasmid and XOFLX® packaging systems (**Figures 2A & 2B**).
- We achieved up to 94% knockdown for anti-BCMA CAR and up to 84% knockdown for anti-CD19 CAR, measured by biotinylated protein-L staining followed by flow cytometry (**Figure 2C**).
- Residual host cell DNA (HCD) in lentiviral vector preparations was quantified by qPCR targeting Alu elements (**Figure 2D**). Residual host cell protein (HCP) was quantified using a semi-quantitative ELISA with anti-HEK293 HRP-conjugated antibody.
- Cargo gene knockdown during production reduced residual HCD (up to 86%) and HCP (up to 37%) in LVV preparations.
- Minimising unwanted byproducts due to production cell death is critical for improving the efficiency and robustness of downstream processing.

***Figure 2**: Performance of conventional and silencing plasmids during LVV production. (A) Cell viability at LVV harvest, (B) Viable cell count at LVV harvest, (C) Chimeric antigen receptor expression at LVV harvest, (D) Residual HCD and HCP in LVV preparations.*

4. LVV Cargo gene silencing increases titres



- LVV production was carried out using three different production platforms, with the transfer plasmid encoding an anti-BCMA CAR.
- LVV particle titre was measured using p24 ELISA. Infectious titre assay was performed by transducing HT1080 cells, followed by gDNA extraction and ddPCR-based VCN quantification, which was then used to calculate infectious titre.
- Knockdown of the anti-BCMA CAR during LVV production increased particle titre by up to 3-fold (**Figure 3A**). It also improved infectious titre in both fully transient and XOFLX™ packaging systems (**Figure 3B**).

***Figure 3**: Anti-BCMA CAR encoding LVV production. (A) Particle titre of anti-BCMA CAR encoding LVV supernatants. (B) Infectious titre of anti-BCMA CAR encoding LVV supernatants.*

5. LVV Cargo gene knockdown facilitates stable producer cell line development

- Constitutive expression of certain cargo genes may limit producer cell growth so a stable cell line cannot be developed.
- We stably integrated either conventional or silenced anti-CD19 CAR transfer plasmids into XOFLX® Packaging cells.
- Silencing the cargo gene enabled generation of stable producer cells that otherwise were unrecoverable (**Figure 4 A-C**).

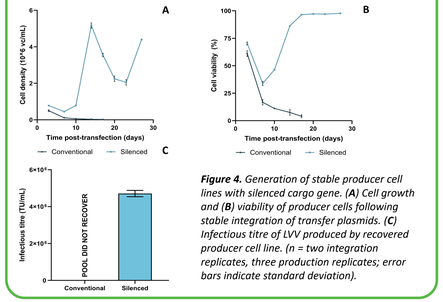


***Figure 4.** Generation of stable producer cell lines with silenced cargo gene. (A) Cell growth and (B) viability of producer cells following stable integration of transfer plasmids. (C) Infectious titre of LVV produced by recovered producer cell line. (n = two integration replicates, three production replicates; error bars indicate standard deviation).*

6. AAV Gene Silencing Technology against EGFP reporter

- shRNA approaches to gene silencing in AAV may lead to PKR activation and translational shutdown.
- Our novel AAV gene silencing technology works with the biology of AAV leading to demonstratable knockdown of reporter genes by fluorescence microscopy (**Figure 5A**) and flow cytometry (**Figure 5B**) without affecting AAV yields (**Figure 5C**).

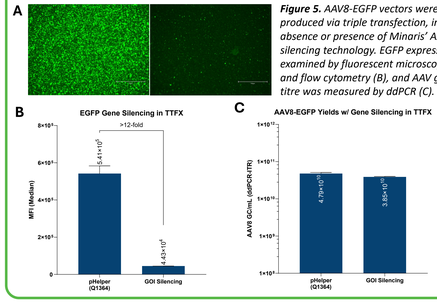


***Figure 5.** AAV8-EGFP vectors were produced via triple transfection, in the absence or presence of Minaris' AAV GOI silencing technology. EGFP expression was examined by fluorescent microscopy (A) and flow cytometry (B), and AAV genome titre was measured by ddPCR (C).*

7. AAV Gene Silencing of Bax during plasmid transfection

- Different variations of the AAV silencing strategy were assessed using a known toxic transgene, BAX (BCL2 associated X, apoptosis regulator).
- Modest improvements (~1.4x) in cell viability during AAV production process (**Figure 6B**) were observed from the two indirect targeting approaches.
- A 16-fold improvement in AAV yield was observed for one strategy (**Figure 6C**).
- This technology has the potential to enable AAV manufacture for previously intractable human transgenes where shRNA-based approaches have been unsuccessful.

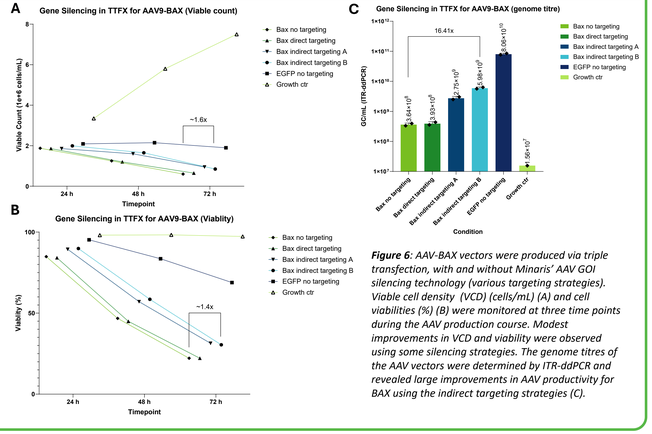


***Figure 6**: AAV-BAX vectors were produced via triple transfection, with and without Minaris' AAV GOI silencing technology (various targeting strategies). Viable cell density (VCD) (cells/mL) (A) and cell viabilities (%) (B) were monitored at three time points during the AAV production course. Modest improvements in VCD and viability were observed using some silencing strategies. The genome titres of the AAV vectors were determined by ITR-ddPCR and revealed large improvements in AAV productivity for BAX using the indirect targeting strategies (C).*

8. Conclusions & Future work

- Cargo gene expression levels were reduced significantly during lentiviral and AAV vector production processes, using different silencing mechanisms developed by Minaris.
- LVV and AAV production yields were maintained (for non-toxic genes) or increased (for cytotoxic transgenes) by implementing the corresponding cargo gene silencing technology.
- Production cell viability was improved, reducing cell death-related process impurities and potentially enabling more efficient and robust downstream process performance.
- The technologies can also facilitate the development of stable producer cell lines for viral vectors encoding cytotoxic cargo genes.
- We will test the silencing mechanisms on a wider range of transgenes in both transient and stable productions.
- Use of these cargo gene silencing systems will de-risk development of novel LVV- and AAV-based therapeutics, by improving production yield and reducing the unwanted protein products from the production process, resulting in decreased manufacturing costs and complexity and improved patient access to LVV- and AAV-based therapies.
- Cargo silencing systems are available for evaluation. Request at www.minaris.com

Corresponding author:
Qian.Liu@minaris.com

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