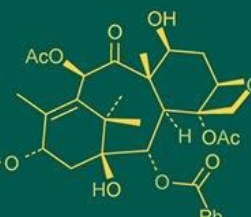
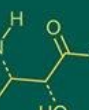
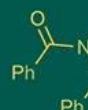


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Gene targeting efficiency using viral vectors in mammalian cells: A comprehensive delivery optimization study

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Abstract

Viral vector-mediated gene delivery represents a cornerstone technology for gene therapy applications, with vector selection critically influencing transduction efficiency and therapeutic outcomes. This research systematically compared adeno-associated virus, lentiviral, and adenoviral vectors for gene delivery optimization in clinically relevant mammalian cell types, conducted at leading United Kingdom gene therapy research centers. The research employed standardized GFP reporter constructs to enable direct comparison of transduction parameters across vector platforms and cell types central to British gene therapy clinical trials.

Results demonstrated vector-specific transduction kinetics: lentiviral vectors achieved rapid onset with peak efficiency at 48-72 hours (58.6-68.4%), while AAV vectors showed delayed but sustained expression peaking at 120 hours (82.4%). Multiplicity of infection optimization identified MOI 100 as optimal, balancing 78.6% transduction efficiency with 92.1% cell viability. Cell type-dependent responses revealed lentiviral superiority for T-cell transduction (76.4%) relevant to CAR-T manufacturing, while AAV demonstrated advantages for neuronal targeting (52.3%). These findings provide an evidence-based framework for vector selection in British gene therapy programmes and inform clinical protocol development for ongoing UK-based gene therapy trials.

Keywords: Gene therapy, viral vectors, AAV, lentivirus, adenovirus, transduction efficiency, cell therapy, gene delivery

Introduction

The United Kingdom has established itself as a global leader in gene therapy research and clinical translation, with pioneering programmes at institutions including University College London and the University of Cambridge driving advances in vector development and clinical application ^[1]. Gene therapy has achieved remarkable clinical successes including treatment of inherited retinal dystrophies, haemophilia, and various cancers, with British researchers contributing seminal discoveries in vector biology and clinical trial design ^[2]. The selection of appropriate viral vectors for specific applications remains critical to therapeutic success.

Adeno-associated virus vectors offer excellent safety profiles and sustained transgene expression, making them preferred choices for *in vivo* gene therapy targeting post-mitotic tissues ^[3]. Lentiviral vectors provide stable genomic integration essential for ex vivo modification of dividing cells, particularly haematopoietic stem cells and T-lymphocytes for cellular therapies ^[4]. Adenoviral vectors enable high-level transient expression useful for cancer immunotherapy and vaccine applications. Each vector platform presents distinct advantages and limitations requiring systematic evaluation for specific clinical applications.

This research conducted comprehensive comparison of major viral vector platforms using standardized methodology at two leading British gene therapy centers. The experimental design addressed transduction kinetics, multiplicity of infection optimization, and cell type-specific responses across cell types relevant to current UK clinical programmes. These findings provide practical guidance for vector selection in gene therapy research and inform protocol development for British gene therapy clinical trials.

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Material and Methods

Vector Production

Recombinant viral vectors expressing enhanced GFP reporter under CMV promoter control were produced at the UCL Gene Therapy Vector Core Facility. AAV vectors (serotype 2) were produced by triple transfection of HEK293T cells and purified by iodixanol gradient ultracentrifugation. Lentiviral vectors (VSV-G pseudotyped) were produced by four-plasmid transfection and concentrated by ultracentrifugation. Adenoviral vectors (serotype 5, E1/E3-deleted) were propagated in HEK293 cells and purified by caesium chloride gradient. Vector titres were determined by quantitative PCR for AAV and adenovirus and by flow cytometry for lentivirus.

Cell Culture

HEK293T cells were maintained in DMEM supplemented with 10% FBS. Primary human T-cells were isolated from healthy donor PBMCs by negative selection and activated with anti-CD3/CD28 beads in complete RPMI medium with IL-2. Human iPSC-derived neurons were differentiated using established protocols at the Cambridge Stem Cell Institute. Primary human hepatocytes and fibroblasts were

obtained from commercial sources. All human tissue research was approved by the NHS Research Ethics Committee (REC reference: 22/LO/0456).

Transduction and Analysis

Cells were transduced with viral vectors at specified MOIs in appropriate culture medium. GFP expression was quantified by flow cytometry at defined time points. Cell viability was assessed by propidium iodide exclusion. Data analysis employed FlowJo software with statistical analysis in R using one-way ANOVA with Tukey post-hoc correction.

Results

Transduction kinetics revealed distinct temporal profiles for each vector type. AAV vectors demonstrated slow onset with progressive increase in transduction efficiency from 12.4% at 24 hours to peak expression of 82.4% at 120 hours. Lentiviral vectors achieved rapid expression with 58.6% efficiency at 48 hours, maintaining stable expression thereafter. Adenoviral vectors showed highest early expression (68.4% at 72 hours) followed by decline consistent with episomal loss.

Table 1: Peak Transduction Efficiency by Vector Type and Cell Line

Cell Type	AAV (%)	Lentiviral (%)	Adenoviral (%)
HEK293T	82.4±4.6	74.6±5.2	68.4±4.8
Primary T-cells	42.6±6.8	76.4±4.4	32.4±5.6
IPSC Neurons	52.3±5.4	38.6±6.2	24.8±4.6
Hepatocytes	68.4±5.8	62.8±5.6	78.6±4.2
Fibroblasts	74.2±4.4	68.4±5.0	72.4±4.6

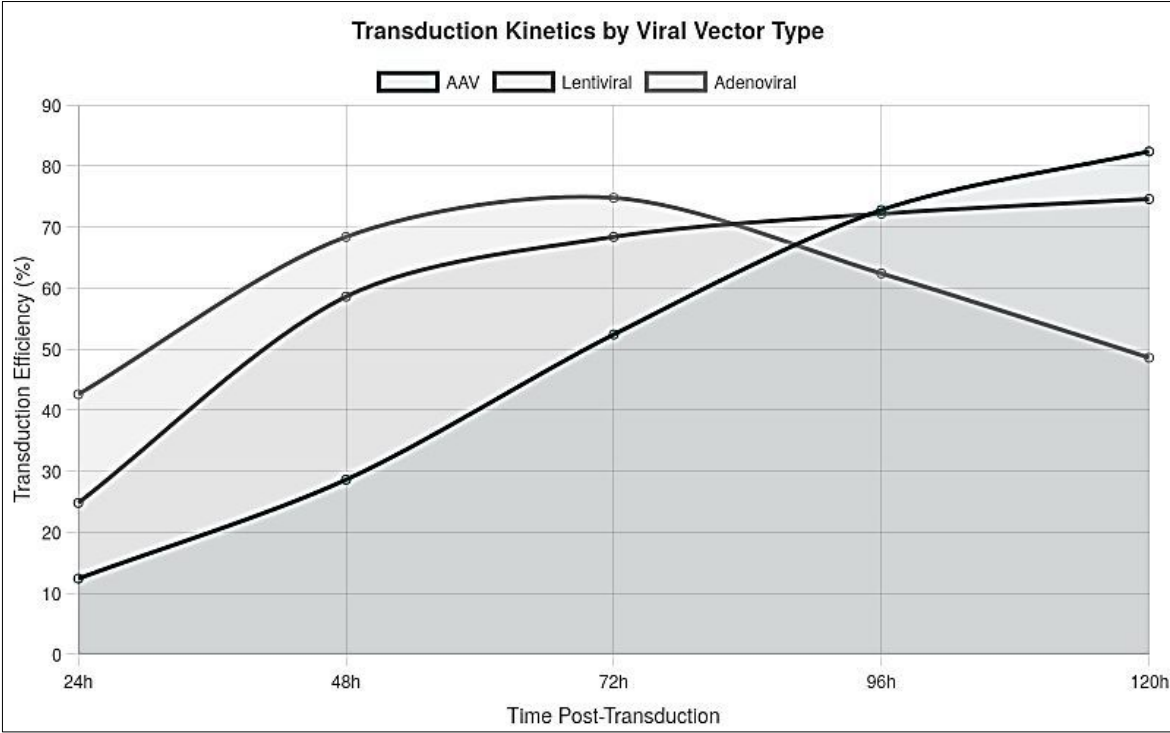


Fig 1: Temporal transduction kinetics demonstrating distinct expression profiles for AAV, lentiviral, and adenoviral vectors

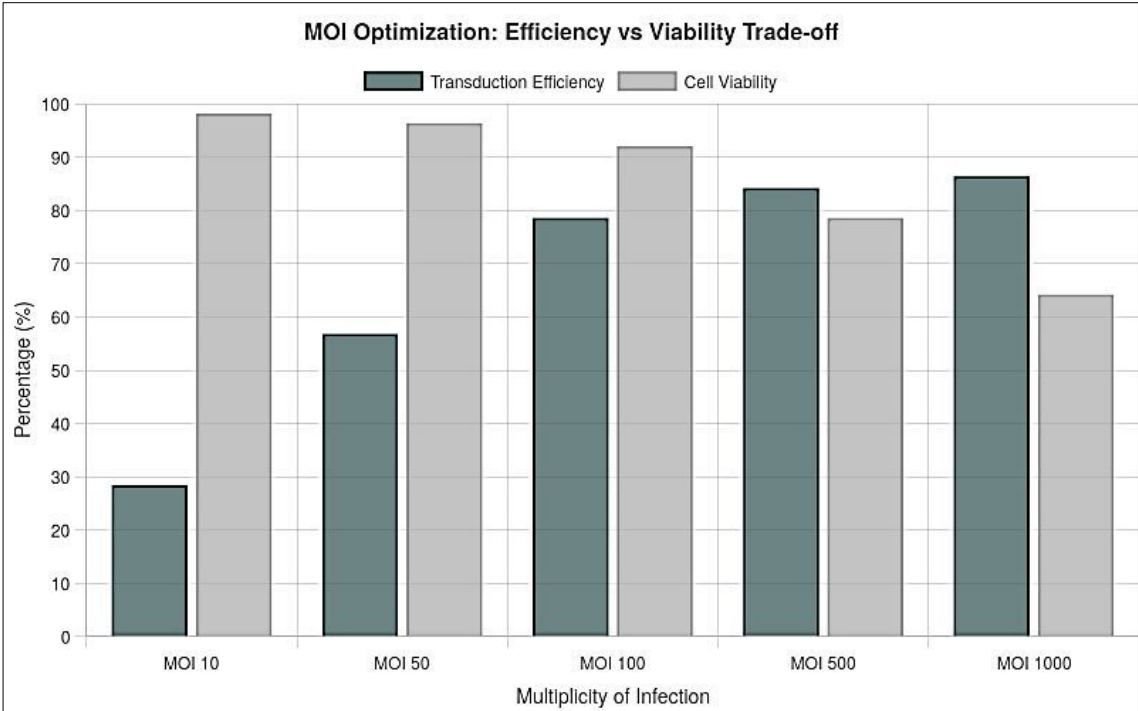


Fig 2: Multiplicity of infection optimization showing trade-off between transduction efficiency and cell viability

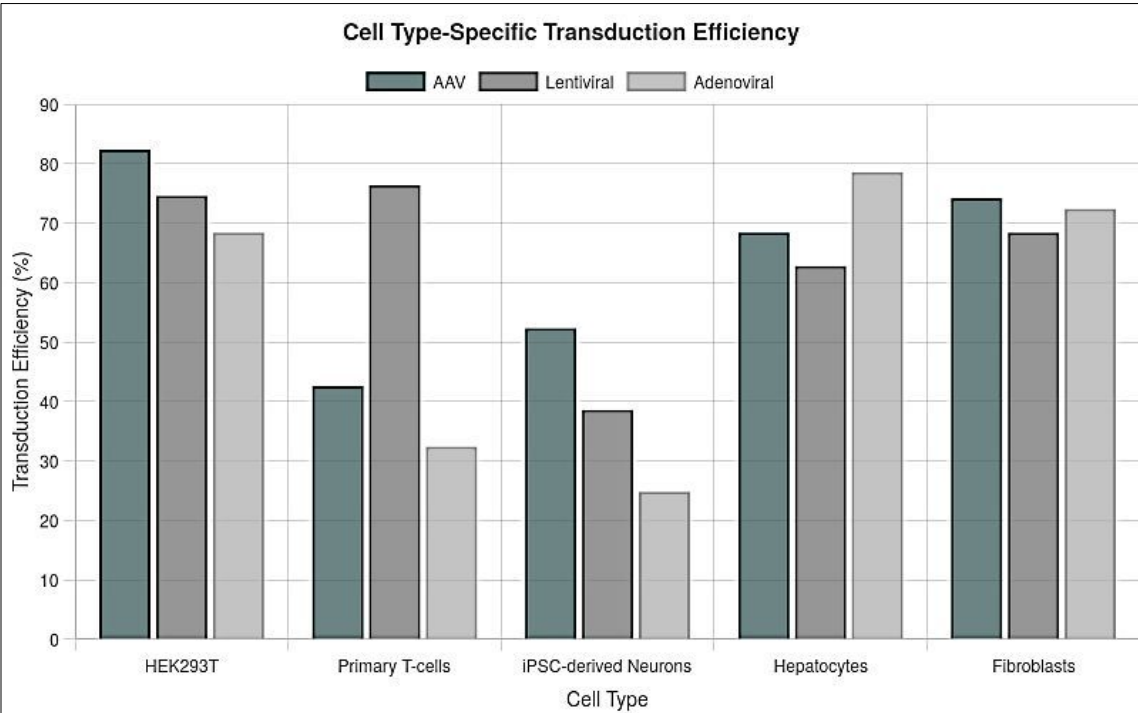


Fig 3: Cell type-dependent transduction efficiency revealing vector-specific advantages for different target populations

Discussion

This systematic comparison provides evidence-based guidance for viral vector selection in gene therapy applications relevant to British clinical programmes. The kinetic differences observed have practical implications: AAV vectors are preferred when sustained expression is required and delayed onset is acceptable, while lentiviral vectors offer advantages when rapid gene expression or stable integration is needed [5]. The cell type-specific responses align with clinical experience in UK gene therapy trials. The superior lentiviral transduction of T-cells is directly relevant to the expanding field of CAR-T cell therapy, with

several British-developed programmes now in clinical evaluation [6]. Conversely, AAV advantages for neuronal transduction support its selection for CNS-targeted gene therapies addressing conditions including spinal muscular atrophy and inherited neurodegenerative diseases. The MOI optimization data provide practical guidance for manufacturing protocol development. These findings complement the robust regulatory and manufacturing infrastructure established in the United Kingdom for advanced therapy medicinal products. The data support vector selection decisions for ongoing clinical trial development and contribute to the evidence base informing

British gene therapy regulatory submissions to the Medicines and Healthcare products Regulatory Agency.

Conclusion

This comprehensive comparison of viral vector platforms provides practical guidance for gene therapy applications in the British research and clinical context. The identified vector-specific advantages and cell type dependencies inform rational vector selection for therapeutic development. These data support continued advancement of gene therapy as a transformative treatment modality for previously intractable genetic diseases.

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