Vector shedding and immunology results from a gene therapy clinical trial for choroideremia

Alun R. Barnard[1,2], Anna Rudenko[1,2], Kanmin Xue[1,2], Robert E. MacLaren[1,2,3].

1. Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford, UK. **2**. Oxford Eye Hospital, Oxford University Hospitals NHS Trust and NIHR Biomedical Research Centre, Oxford, UK.
3. Moorfields Eye Hospital Foundation Trust & UCL Institute of Ophthalmology NIHR Biomedical Research Centre, London, UK.

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Background & Purpose

Assessing the safety profile of gene therapy treatment often involves monitoring for systemic immunological responses to the delivery of the vector and gathering data on how vector disseminates into the environment through secretions and/or excreta of the patient (defined as vector shedding). We report here on vector shedding and systemic immune responses in 5 patients who received subretinal injection of an AAV-based vector (1E+11 genome particles) as part of a clinical trial of gene therapy for choroideremia[1,2] (ClinicalTrials.gov: identifier: NCT01461213).

Methods

Vector shedding and immunology samples were collected at pre-operative baseline, 1 day, 1 week, 1 month and 3 months post-surgery (Fig 1). Samples of saliva, urine, blood, and tears were collected according to recently published methods[3] and assessed for the presence of AAV-genomes by validated, quantitative PCR analysis (Covance Laboratories, Harrogate, UK; limit of detection: 50 genomes per 5 µl). At month 6, only a blood sample was taken for immunology. All serum samples were assessed for systemic immune responses by analysing for the presence of neutralising antibodies against AAV2 using a validated, in vitro reporter system (Genosafe, Paris, France).

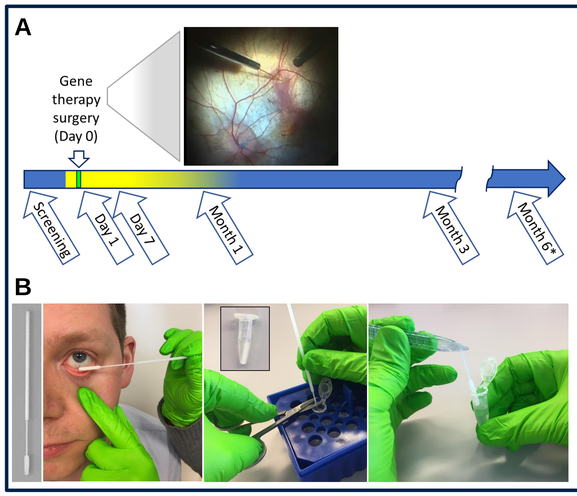

Figure 1: Sample collection. *A. Schematic showing timing of sampling collection under the protocol. *Vector shedding samples not collected. Green vertical bar shows gene therapy surgery timing. Yellow shading indicates a tapering dose of oral prednisolone for immunosuppression/anti-inflammation. Inset image shows fundus view of choroideremia patient undergoing surgery.* ***B.*** *Images illustrating the materials needed and techniques followed to collect a tear sample (adapted from Barnard et al., 2018).*

The peripheral blood mononuclear cell (PBMC) fraction was isolated using Leucosep tubes pre-filled with Ficoll Paque PLUS and promptly cryopreserved (FBS with 10% DMSO). These samples were later analysed for ex vivo cell mediated immune responses using a validated, Interferon-gamma (IFN-γ) Enzyme-linked immunosorbent spot (ELISPOT) assay (Covance Laboratories, Harrogate, UK). T-cell activation (spot formation) was assessed after >24 hour exposure to one of several treatments:

- *REP1 mimotopes (Pool 1-3)*, a 214 library of 15-mer peptides, mimicking potential epitopes (mimotopes with 12 peptide overlap) was combined in 3 serial pools (mode of 72 peptides).
- *AAV2*, intact capsids of AAV2, the same vector as used in the trial.
- *CEF mimotope pool*, 23 MHC class I-restricted T-cell epitopes from human CMV, EBV and influenza (flu) viruses, designed as an antigen-specific positive control to stimulate IFN-γ responses in CD8+ T cells from donors with a variety of HLA types and work in almost 90% of all Caucasians.
- *Media*, negative control wells contained medium without any antigen/stimulant.

Results

Shedding of gene therapy vector

A positive vector shedding result was found in the tear sample of patient 110, collected from the treated eye on the day immediately following surgery. All other samples and timepoints for this patient were negative, as were all from the other patients (Table 1).

Table 1: Assessment of presence of vector genomes in study samples

			Sample Type (Copies/5 µL)				
Patient ID	Dosed Eye	Time Point	Blood	Saliva	Tears Left Eye (OS)	Tears Right Eye (OD)	Urine
110/RC56	OD	screening	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 1	<50.0	<50.0	<50.0	920.9	<50.0
		Day 7	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 3	<50.0	<50.0	<50.0	<50.0	<50.0
111/GW43	OD	Pre-dose/screening	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 7	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 3	<50.0	<50.0	<50.0	<50.0	<50.0
112/IB74	OD	Pre-dose/screening	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 7	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 3	<50.0	<50.0	<50.0	<50.0	<50.0
113/PA60	OS	Pre-dose/screening	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 7	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 3	<50.0	<50.0	<50.0	<50.0	<50.0
114/JP91	OD	Pre-dose/screening	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 7	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 3	<50.0	<50.0	<50.0	<50.0	<50.0

Immune responses following AAV2-REP1 gene therapy

Neutralising, anti-AAV2 antibodies in serum samples were low in all 5 patients at baseline (titre <10). Importantly, these did not rise after treatment with the vector and remained <10 throughout the follow-up period, indicating that no significant humoral immunological responses were elicited in response to delivery of the gene therapy (AAV2-REP1) vector at this dose (Table 2).

Table 2: AAV2 neutralising antibodies titres in serum samples over time.

		Anti-AAV2 NAb at each time point (titre)					
Patient ID	Dose	Screening	Day 1	Day 7	Month 1	Month 3	Month 6
110/RC56	High	<10	<10	<10	<10	<10	<10
111/GW43	High	<10	<10	<10	<10	<10	<10
112/IB74	High	<10	<10	<10	<10	<10	<10
113/PA60	High	<10	<10	<10	<10	<10	<10
114/JP91	High	<10	<10	<10	<10	<10	<10

Values shown are the reciprocal of the dilution needed to obtain a negative response with the sample.

In patients 110-112, cellular responses to REP1 mimotope pools and AAV remained negative (<3x media control) throughout, although individuals responded differentially to the positive control (Fig 2). Patient 113 had a positive response to REP1 mimotope pool 1 at baseline but, as this tested negative throughout the follow-up, the significance of this finding is difficult to interpret. Patient 114 had positive responses to REP1 pool 1 on day 7 and month 1 and to pool 3 on month 1 only. Although These were mild positive response (3-4x media control) and returned to negative responses at months 3 and 6. Thus, positive responses in this patient did not appear to be clinically meaningful and were not long lasting.

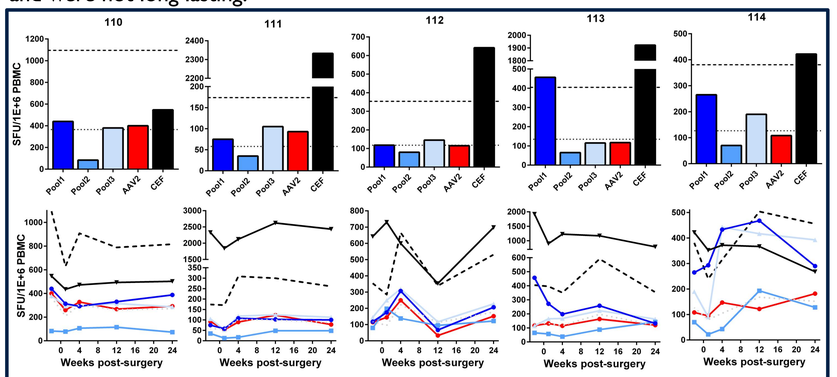

Figure 2: ELISPOT assay results for PBMC samples. *Top row shows response from patients at baseline, when cells were challenged to different REP1 mimotope pools, AAV virus or positive control CEF mimotope pool. Single values are shown. Grey dotted line shows control (media), glack dashed line represents 3x negative control, The bottom row shows the responses across the follow up period. REP1 mimotope pool 1(-●-), pool 2(-■-), pool 3(-▲-); AAV2 virus (-●-); CEF mimotope pool (-▼-).*

Conclusion

Our results are similar to others, which have shown very limited vector shedding and a lack of clinically meaningful systemic responses to the small AAV2 doses commonly used in retinal gene therapy. The negative results demonstrate that the surgical delivery technique used here avoids excessive vector shedding and limits immune responses by exploiting the immune privileged environment of the subretinal space.

References & Disclosure

1. MacLaren RE, Groppe M, Barnard AR, et al., Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. *Lancet*. 2014; 383(9923):1129-37.
2. Edwards TL, Jolly JK, Groppe M, Barnard AR, et al. Visual Acuity after Retinal Gene Therapy for Choroideremia. *N Engl J Med*. 2016; 374(20):1996-8
3. Barnard AR, Rudenko AN, MacLaren RE. Vector Shedding and Immunogenicity Sampling for Retinal Gene Therapy. *Methods Mol Biol*. 2018; 1715:359-371

REM is a named co-inventor on UK patent application 1103062.4, filed in Feb 2011 and owned by the University of Oxford. REM is also a Director and Board Member of Nightstar Therapeutics (Welcome Trust Building, 215 Euston Road, London UK), a retinal gene therapy company established by the University of Oxford and funded by the Wellcome Trust.

Nuffield Department of Clinical Neurosciences
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alun.barnard@eye.ox.ac.uk