CASE STUDY | GENE THERAPY | KIDNEY

De-Risking AAV Gene Therapy Delivery for Polycystic Kidney Disease Using Ex Vivo Human Kidney Perfusion

Mapping global and cell targeted vector distribution across intact human kidneys

Customer Snapshot

Torque Bio leveraged Revalia's ex vivo Human Kidney Perfusion Platform to improve and better understand delivery of its novel kidney optimized AAV-based gene therapy for polycystic kidney disease. The platform enabled direct visualization and assessment of vector biodistribution and transduction across multiple intact human kidneys, in addition to supporting continued translational development and optimization of parameters for Torque's upcoming first-in-human clinical study.

THE ENGAGEMENT

Customer: Torque Bio, a biotech poised to enter clinical development and finalizing plans for its upcoming first-in-human clinical trials

Program: AAV-based gene therapy for polycystic kidney disease using AAV.k20, a novel capsid optimized for human kidney transduction

Revalia offering: Kidney Delivery and Distribution Study, intact perfused human donor kidneys

Perfusion window: Up to 72 hours per organ, with continuous physiologic monitoring

The Challenge

Before advancing its lead ADPKD program to the clinic, Torque Bio needed to address two critical questions:

- **Translation to humans:** Does the novel kidney-optimized AAV.k20 capsid and local kidney delivery approach evaluated in large-animal models translate to the anatomy and physiology of an intact human kidney?
- **Delivery optimization:** Can dosing and delivery parameters be optimized to achieve broad vector biodistribution and efficient transduction of target cells?

Existing human cell and organoid models provide supportive data to address these key questions, but they lack the native and complex three-dimensional architecture, vascular networks, and integrated physiology of an intact kidney. These limitations prevent accurate assessment of whole-organ functions, including vascular hemodynamics, autoregulation, and filtration, as well as vector biodistribution and cell-specific targeting.

Revalia's ex vivo Human Kidney Perfusion Platform addressed this critical translational gap by enabling Torque Bio to evaluate and optimize AAV.k20 gene therapy delivery in intact human kidneys, reducing development risk before costly downstream studies while generating human-relevant data prior to entering the clinic.

The Approach

Revalia's intact, perfused human donor kidney platform allowed Torque Bio to test local administration of their novel AAV.k20 capsid evolved for human renal tubule cell transduction directly in human tissue, with a level of control and sampling that is not possible in the clinic or in animal models.

Each kidney was maintained on normothermic machine perfusion for 72 hours. Vector was administered under real-time fluoroscopic imaging, with contrast co-administered so the team could watch distribution as it happened, in real-time. Donor-matched pre-treatment biopsies from the same organ served as internal controls. Physiologic parameters were tracked continuously through dosing and across the full perfusion window to ensure the organ remained viable.

Revalia's Kidney Delivery and Distribution Study

What was measured	How
Distribution during dosing	Real-time 2D fluoroscopy of vector during initial delivery
Organ viability and function	Continuous renal artery pressure and flow, glucose handling, and oxygen extraction across 72 hours
Spatial vector biodistribution and transgene expression	Dual fluorescent in *situ* hybridization (FISH) via RNAscope to simultaneously detect vector DNA and transgene RNA within the same tissue section, enabling quantification of vector biodistribution and transduction across the tissue and within specific target cell populations

The Data

1. Real time visualization of widespread vector distribution during administration

The AAV.k20 vector was mixed with a contrast agent so that distribution could be visualized and confirmed in real time using 2D-fluoroscopy. Notably, in the example below, the vector/contrast mixture effectively exited the renal pelvis and distributed broadly into the surrounding tissue, with no evidence of leakage from the administration site.

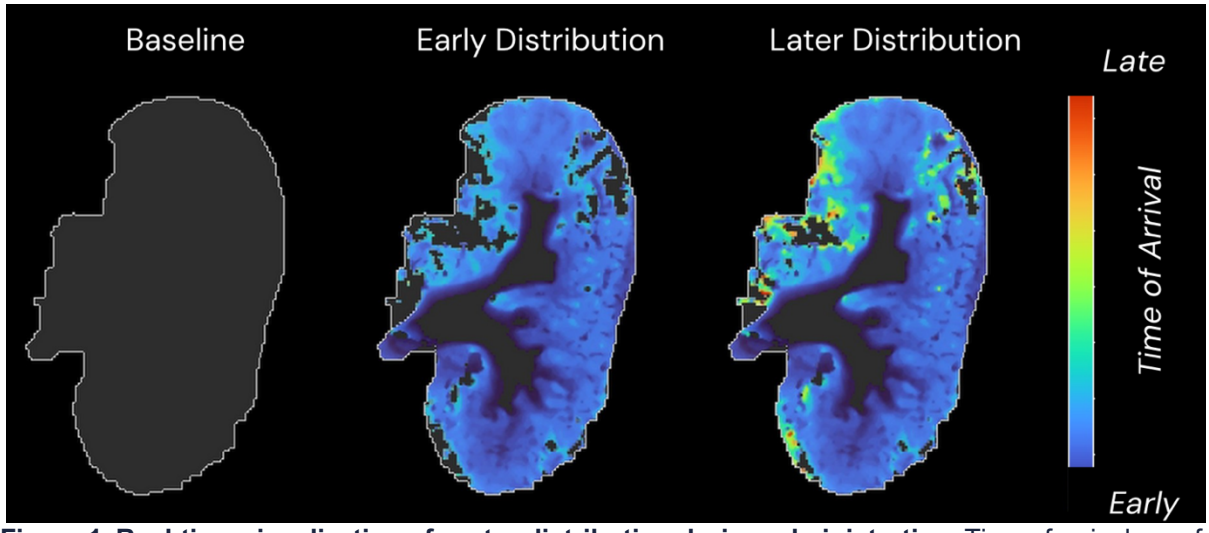


Figure 1. Real-time visualization of vector distribution during administration. Time of arrival map from real-time 2D fluoroscopy with noise-corrected baseline subtraction, shown at the start of administration, mid-infusion, and at completion showing that the vector/contrast mixture fills the renal pelvis and distributes outward through the collecting system.

2. Kidneys remained functional over three days

Revalia's kidney platform maintains organ viability for 72 hours, with AAV.k20 vector administration having no apparent impact on kidney function. In the representative example shown, renal artery pressure and flow, glucose handling, and oxygen extraction (blue lines) stabilized early in perfusion

and remained within the reference control range (gray shading) throughout the 72-hour study, with no appreciable changes following vector administration. These findings demonstrate that the vector delivery and subsequent biodistribution and transduction results were generated in viable, functioning human kidneys.

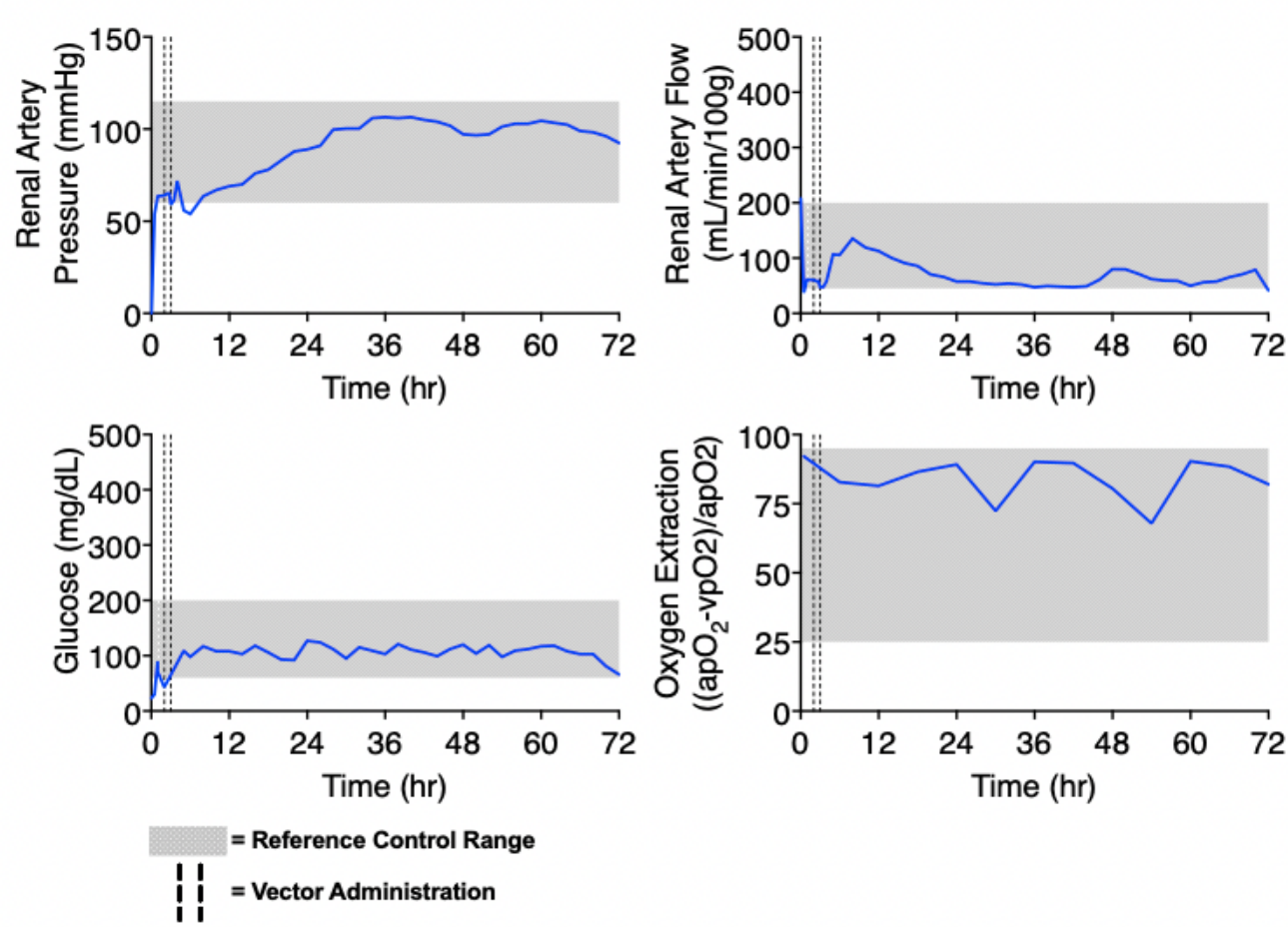


Figure 2. AAV.k20 vector delivery does not influence kidney viability and function over 72-hours of perfusion. Renal artery pressure, renal artery flow, glucose, and oxygen extraction from a representative kidney administered the vector between 2 and 3 hours of perfusion (dashed vertical lines). The shaded band indicates the reference control range.

3. Transduction localized to the intended cell populations

Dual fluorescent in *situ* hybridization (FISH) using DNA- and RNA-targeting probes via the RNAscope platform from expansive tissue biopsies allowed for quantification and visualization of the AAV.k20 vector and transgene at single-cell resolution from all regions of the kidney. The signal was widespread and localized to tubule epithelial cells including proximal tubule, distal tubule, and collecting duct cells,

which are target cell types for the treatment ADPKD and where the AAV.k20 vector is designed to reach.

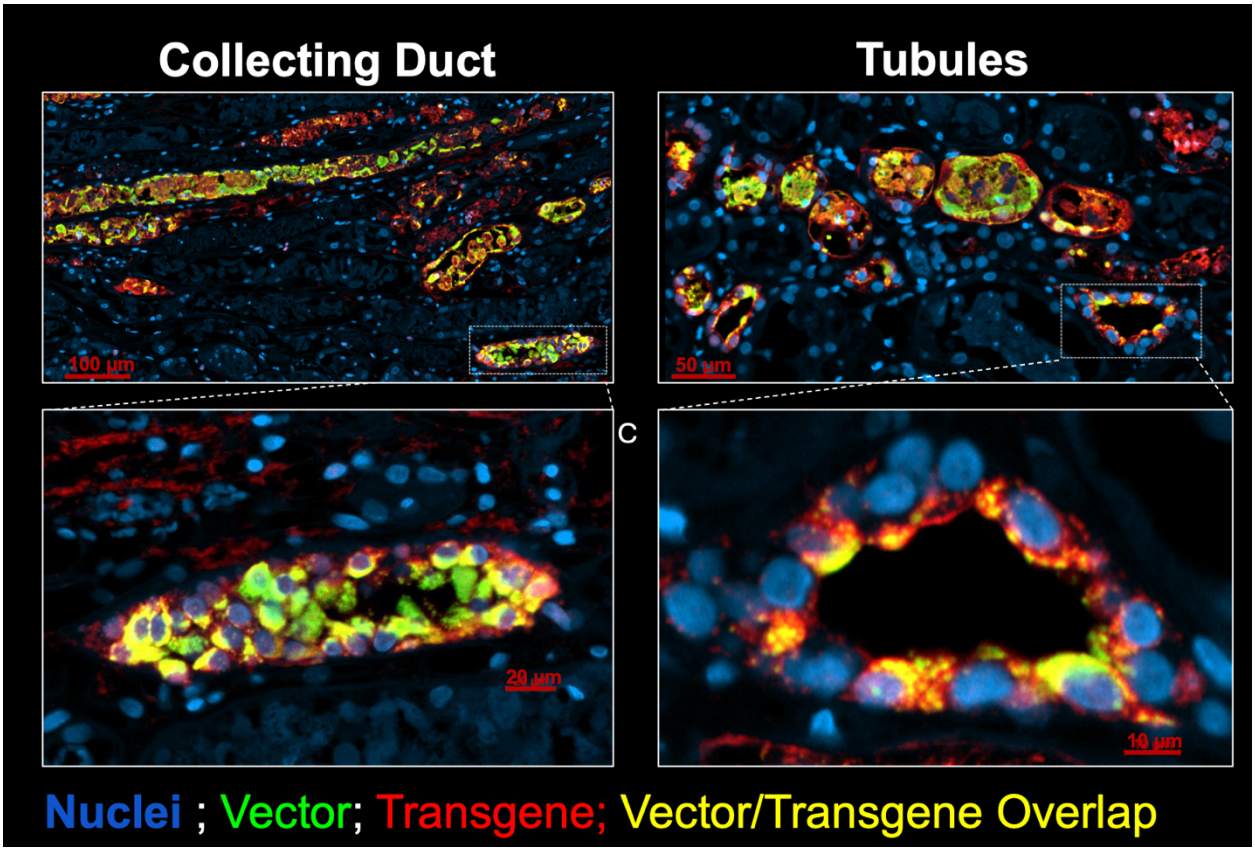


Figure 3. Cell-level vector uptake and transgene localization. Dual FISH via RNAscope across all regions of the kidney allowed for visualization of vector DNA uptake and transgene RNA expression in collecting ducts and tubules. DAPI in blue, vector DNA in green, transgene RNA in red, vector/transgene overlap in yellow. Insets show increasing magnification.

The Result

The data package gave Torque Bio direct evidence, in real human kidney tissue, that their human kidney-optimized AAV.k20 capsid and dosing approach achieves broad vector distribution and transduction of target cells across an entire kidney, without compromising organ function. Torque used this data package to refine its large-animal translational study design and materially de-risk progress to first-in-human clinical studies.

> “The ability to evaluate the AAV.k20 human kidney-optimized capsid and localized delivery approach directly in intact human donor kidneys, through our collaboration with Revalia Bio, provides an important translational bridge as we finalize our preparations to enter the clinic. These findings complement our existing human organoid and large-animal data and further support the differentiation of the AAV.k20 capsid, now demonstrated directly in intact human kidneys.”
>
> Amy Rhoden Smith, Vice President, Research, Torque Bio

What Else This Applies To

The study design is not specific to AAV or to polycystic kidney disease. The same framework of intact human organ, dose-and-sample, donor-matched controls, dense regional biopsy, and paired distribution and transduction readouts applies directly to:

- Other kidney-directed modalities, including oligonucleotide, siRNA, mRNA-LNP, and small molecule programs, substituting the relevant detection assay
- Other delivery routes in the kidney, including intra-arterial bolus or infusion, intravenous bolus or infusion, direct parenchymal administration or other clinically translatable administration techniques.
- Other question types on the same platform, including early efficacy and toxicity studies

WORKING WITH REVALIA

Revalia Bio maintains intact, perfused human donor organs for pharmaceutical research. Sponsors dose their own molecules in functioning human organs and get spatial, functional, and molecular data that animal models and in vitro systems cannot produce.

For more details, visit us @ www.revaliabio.com.