CASE STUDY | DRUG INDUCED KIDNEY INJURY | KIDNEY

A Human Model for Drug-Induced Kidney Injury Using Ex Vivo Human Kidney Perfusion

A platform for testing drug induced toxicity and novel compounds targeting acute kidney injury

Study Snapshot

Revalia Bio is the technical lead for an up to $26.7M contract awarded under the Advanced Research Projects Agency for Health (ARPA-H) Computational ADME-Tox and Physiology Analysis for Safer Therapeutics (CATALYST) program. The project, titled *An Integrated Human Data Approach to Unlock the Future of In Silico Learning Models,* supports Revalia's mission to redefine how drug pharmacokinetics and safety are predicted before clinical trials by generating human relevant data from ex vivo perfused human kidney and liver for predictive machine learning (ML) and artificial intelligence (AI) models. Using cisplatin, a chemotherapeutic agent with well-established nephrotoxic effects, Revalia demonstrated the platform's ability to detect drug-induced acute kidney injury at the tissue, cellular, and molecular levels across multiple functioning human kidneys. These findings, together with data being generated using additional nephrotoxic compounds are supporting the development of ML and AI models aimed at predicting drug-induced nephrotoxicity of novel compounds. Furthermore, the consistent activation of acute kidney injury-associated pathways across donors establishes a foundation for using this human kidney toxicity model to evaluate novel acute kidney injury treatments.

THE ENGAGEMENT

Customer: Advanced Research Projects Agency for Health (ARPAH), a federal research agency sponsoring a research program focused on Computational ADME-TOX and Physiology Analysis for Safer Therapeutics (CATALYST).

Program: Cisplatin-induced kidney injury for acute nephrotoxicity disease modeling

Revalia offering: Kidney Toxicity Study, functional perfused human donor kidneys

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

This research was funded, in part, by the Advanced Research Projects Agency for Health (ARPA-H) under Agreement No. 1AY2AX000118. The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the official policies, either expressed or implied, of the U.S. Government.

The Challenge

Cisplatin is an effective cancer treatment, but kidney toxicity limits its use. In one clinical cohort, 31.5% of adults developed acute kidney injury after their first treatment cycle [1]. Despite its substantial clinical burden, there are no approved therapies that directly reverse drug-induced acute kidney injury or promote kidney recovery following acute kidney injury [2,3].

Animal models and human organoids can reproduce aspects of cisplatin-induced acute kidney injury, but species differences and incomplete organoid maturation limit their ability to capture the

integrated tissue, cellular, and molecular responses of an intact human kidney [4-6]. These limitations led us to ask two questions:

- Can a functional human ex vivo kidney perfusion model reproduces the established tissue and molecular effects of cisplatin?

- Can donor-matched paired kidneys, with one kidney receiving cisplatin and the contralateral kidney receiving vehicle, control for donor-to-donor variability in cisplatin-induced nephrotoxic responses?

Revalia's ex vivo Human Kidney Perfusion Platform addresses this critical translational gap by reproducing cisplatin-induced kidney injury in functional human kidneys and enabling comprehensive assessment of physiological dysfunction, cellular injury, and molecular responses. These findings demonstrate the platform's ability to detect known drug-induced nephrotoxicity across multiple biological endpoints. Furthermore, these data, together with findings from additional drugs with known and distinct mechanisms of nephrotoxicity, are being used to map toxicity responses across multiple orthogonal biological endpoints to provide a new corpus of previously unavailable human data to support the development of advanced ML and AI models to predict the nephrotoxicity of novel compounds. Finally, the consistent activation of acute kidney injury-associated pathways across donors provides a foundation for using this human kidney platform to evaluate novel therapeutic interventions for acute kidney injury.

The Approach

Ten kidneys from five donors were perfused for 72 hours. Within each kidney pair from the same donor, one kidney received a 60 mg arterial bolus of cisplatin and the other received vehicle. The cisplatin dose was selected based on findings from large-animal models of acute kidney injury, in which comparable single-bolus doses of cisplatin produced renal dysfunction and tubular injury within 48-72 hours [7-8].
In this ex vivo perfusion study, longitudinal tissue biopsies and biofluid sampling were performed to assess histopathology, cell death, circulating biomarkers, and transcriptomic responses.

Revalia's Kidney Toxicity Study

What was measured	How
Tissue injury and cell death	Longitudinal tissue biopsies for histopathological assessment and quantification of cell death using TUNEL staining.
Circulating injury and inflammatory biomarkers	Serial perfusate sampling to quantify markers of kidney injury, cellular stress, and inflammation over time.
Acute kidney injury-associated molecular pathways	Longitudinal RNA sequencing of tissue biopsies to identify differentially expressed genes and pathways associated with cisplatin-induced acute kidney injury.

The Data

1. Cisplatin produced clinically relevant tissue level damage in a living human kidney

Cisplatin exposure led to a significant increase in kidney injury, as determined by histopathological quantification of longitudinal tissue biopsies (Left Panel). As illustrated in representative histological images, this injury was characterized by hallmark features of cisplatin-induced acute kidney injury, including acute tubular necrosis and loss of tubular epithelial cells (Right Panel). Together, these findings demonstrate that cisplatin exposure induces quantifiable histopathological injury in *ex vivo* perfused human kidneys, consistent with key features of cisplatin-induced AKI [4].

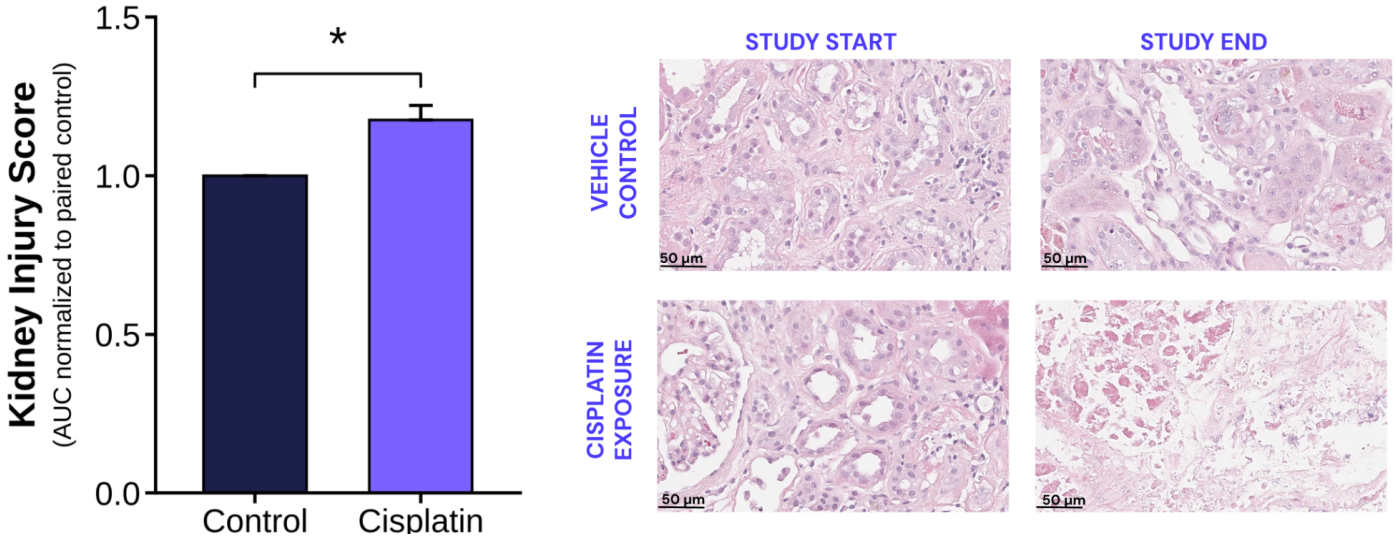


Figure 1. Cisplatin exposure induces kidney injury characterized by hallmark histopathological features of acute kidney injury. ***Left panel*****:** Kidney injury scores were determined by histopathological scoring of hematoxylin and eosin (H&E)-stained tissue sections collected longitudinally over 72 hours from five donor-matched kidney pairs (n = 10 kidneys). Data are expressed as area under the curve (AUC) relative to the paired vehicle control, following normalization to the pre-dose baseline at 6 hours. ***Right panel:*** Representative histological images of vehicle control and cisplatin-exposed kidneys at the beginning and end of perfusion, demonstrating preserved tubular architecture in control tissue and acute tubular necrosis and tubular epithelial cell loss in cisplatin-exposed tissue. Statistical differences in AUC were assessed using paired, two-tailed Student's t-tests ($p < 0.05$).

2. Cisplatin exposure induces progressive cell death in perfused human kidneys, consistent with a key mechanism of cisplatin-induced acute kidney injury

Mechanistically, cisplatin induces cell death in both tumor and non-tumor cells through DNA damage. Using serial tissue biopsies and quantitative imaging, we demonstrated that cisplatin exposure induced a significant, progressive increase in cell death via TUNEL staining over the course of the perfusion across five paired kidney donors. These findings are consistent with a key

mechanism of cisplatin-induced acute kidney injury and further support the clinical relevance of this human kidney model [4,9,10].

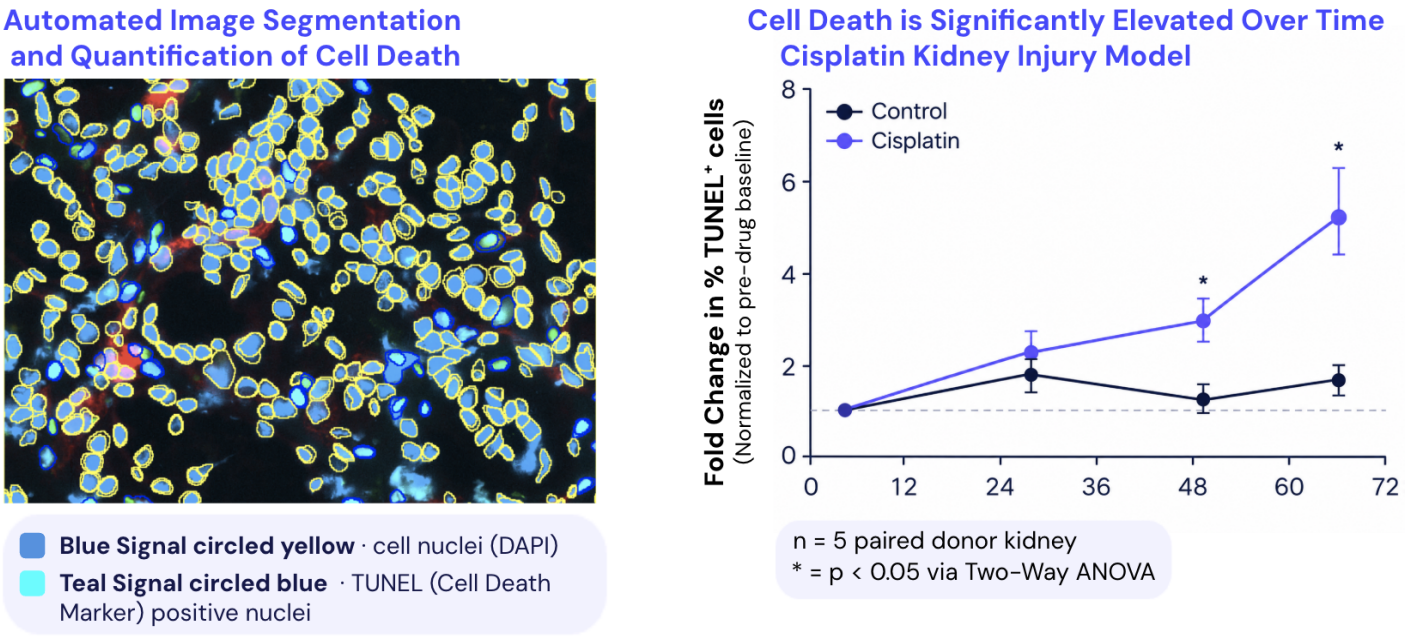


Figure 2. Cisplatin exposure progressively increases cell death over time, recapitulating a key mechanism of cisplatin mediated acute kidney injury. ***Left panel:*** Representative immunofluorescence image illustrating automated cell segmentation and quantification. Total nuclei are stained blue with DAPI and outlined in yellow, while TUNEL-positive nuclei, indicating cell death, are stained teal and outlined in yellow. ***Right panel:*** Quantification of cell death using TUNEL staining in serial tissue biopsies collected during extended perfusion across five paired kidney donors treated with cisplatin or vehicle (n = 10 kidneys). Cell death was calculated as the percentage of TUNEL-positive nuclei relative to total nuclei and normalized to pre-dose baseline levels at 6 hours.

3. Cisplatin exposure increases circulating pro-inflammatory cytokines associated with acute kidney injury.

Cisplatin exposure increased circulating levels of IL-1β, TNF-α, and IL-6 in perfused human kidneys. These pro-inflammatory cytokines are associated with the inflammatory response to acute kidney injury across multiple etiologies and have also been implicated in cisplatin-induced nephrotoxicity. Together, these findings demonstrate that the model captures an inflammatory component of kidney injury, further supporting its relevance for studying acute kidney injury [4, 11].

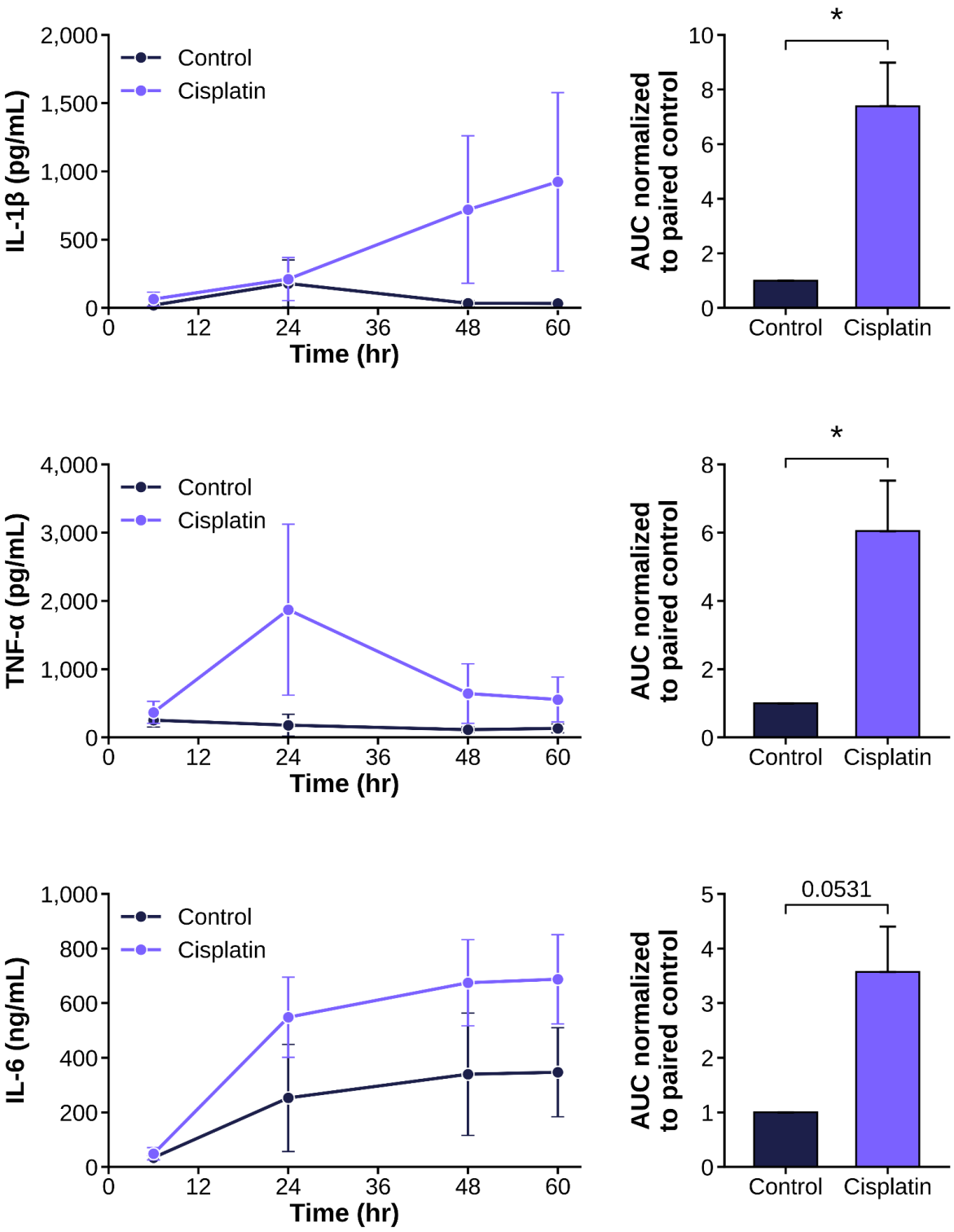


Figure 3. Cisplatin exposure elevates circulating levels of pro-inflammatory cytokines associated with acute kidney injury. A multiplex immunoassay (MSD) was used to quantify IL-1β, TNF-α, and IL-6 in serial arterial perfusate samples collected during extended perfusion of four paired donor kidneys treated with cisplatin or vehicle (n = 8 kidneys, n=4 donor pairs). Data are presented as absolute cytokine concentrations over time (line graphs) and area under the curve (AUC) normalized to the corresponding donor-matched vehicle control (bar graphs). Statistical differences in AUC were assessed using paired, two-tailed Student's t-tests (*$p < 0.05$; $p = 0.0531$ for IL-6).

4. **Cisplatin exposure upregulates known acute injury pathways and donor responses were highly concordant**

Temporal bulk RNA sequencing was performed on serially collected kidney tissue samples throughout the perfusions. Pathway analysis of differentially expressed genes revealed significant elevation in classical pathways associated with acute kidney injury (Left Panel). Notably, temporal gene expression analysis demonstrated highly consistent responses across donors for the most strongly upregulated genes within these pathways. MDM2, a downstream target of p53, highlights this consistent response across donors (Right Panel) [4, 9].

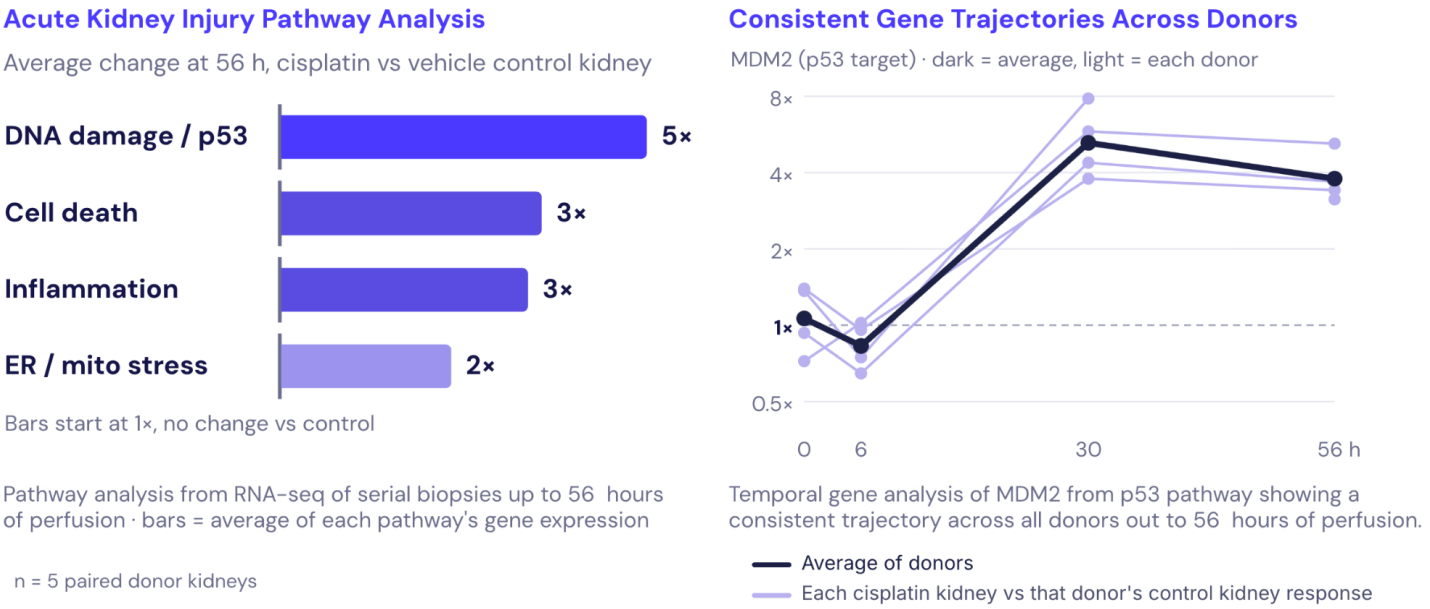


Figure 4. Cisplatin exposure triggers known acute kidney injury pathways, and donor responses were highly consistent. ***Left panel***: Bulk RNA sequencing was performed on serial tissue samples collected during extended perfusion of five paired donor kidneys treated with cisplatin or vehicle (n = 10 kidneys). Pathway enrichment analysis was performed on differentially expressed genes between cisplatin-treated kidneys and their matched controls. Significant pathway changes are shown as normalized enrichment scores. ***Right panel:*** Temporal expression trajectories of MDM2, a downstream target of p53, in cisplatin-treated kidneys relative to their respective matched controls. Individual donor responses are shown in light purple, with the mean response across donors shown in black.

The Result

The Revalia Kidney Toxicity Platform demonstrated the ability to identify cisplatin-induced nephrotoxicity across multiple data layers, linking drug exposure to tissue injury, inflammatory responses, and molecular signatures in intact human kidneys. These findings, together with data generated using additional nephrotoxic compounds as part of the ARPAH CATALYST award, will provide a new corpus of previously unavailable human data to support the development of advanced ML and AI models to predict the nephrotoxicity of novel compounds. Furthermore, the consistent activation of acute kidney injury-associated pathways across donors establishes a foundation for using this human kidney injury model to evaluate potential acute kidney injury treatments.

What Else This Applies To

The study design and experimental framework extend beyond cisplatin and can be applied to a broad range of compounds, therapeutic modalities, and research objectives using intact human kidneys. These applications include:

- **Additional nephrotoxic compounds:** Evaluation of known nephrotoxic agents, including bevacizumab, tacrolimus, gentamicin, and polymyxin B, all of which are actively being studied at Revalia.
- **Diverse therapeutic modalities:** Safety and toxicity assessment of small molecules, biologics, viral vector-based gene therapies, oligonucleotides, siRNA, and mRNA-lipid nanoparticles (LNPs).
- **Multiple administration routes:** Evaluation of clinically relevant delivery approaches, including intra-arterial infusion, intravenous bolus or infusion, retrograde ureteral administration, direct parenchymal injection, and other translatable delivery techniques.
- **Expanded research applications:** Beyond toxicity assessment, the platform supports studies of therapeutic delivery and biodistribution and early efficacy.

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.

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