# A postmortem whole human brain platform for CNS drug discovery

Congwei Wang, Nghia Nguyen, Scott Pope, Machele Riccio, Josip Butkovic, Ivan Mumlek, Chunmin Ge, Raghav Kansal, Leah Rosales, Lindsay Slupczewski, Lucija Barisic, Brad Parry, Sean Murphy, Zvonimir Vrselja, Paul D. Wes
Bexorg, New Haven, CT, USA

## INTRODUCTION

A major barrier to developing effective therapies for Alzheimer's disease is the limited ability of preclinical models to predict clinical efficacy. Animal models often fail to recapitulate key aspects of human disease biology and etiology, and human-derived in vitro systems, while valuable, typically lack the multicellular architecture and mature phenotypes of the adult aged brain. To address this gap, we established BrainEx, an ex vivo whole brain perfusion platform that supports physiological maintenance of molecular and cellular function in postmortem human brains, including tissue from donors with Alzheimer's disease. BrainEx enables preclinical drug discovery and translational validation directly in the human disease brain, supporting target validation, pharmacokinetic assessment and brain penetration, pharmacodynamic and functional readouts, biomarker discovery, and novel target identification.

## ETHICAL CONSIDERATIONS

Acquisition of postmortem human brains adheres to the highest possible ethical standards, overseen by an independent board of world-renowned bioethicists. Brains are procured through Organ Procurement Organizations (OPOs) with enhanced levels of consent from patients and families that specifically cover the BrainEx platform. Measures are taken on the BrainEx device to ensure that there is no possibility of coordinated network activity associated with consciousness.

## [1] THE BRAINEX PLATFORM

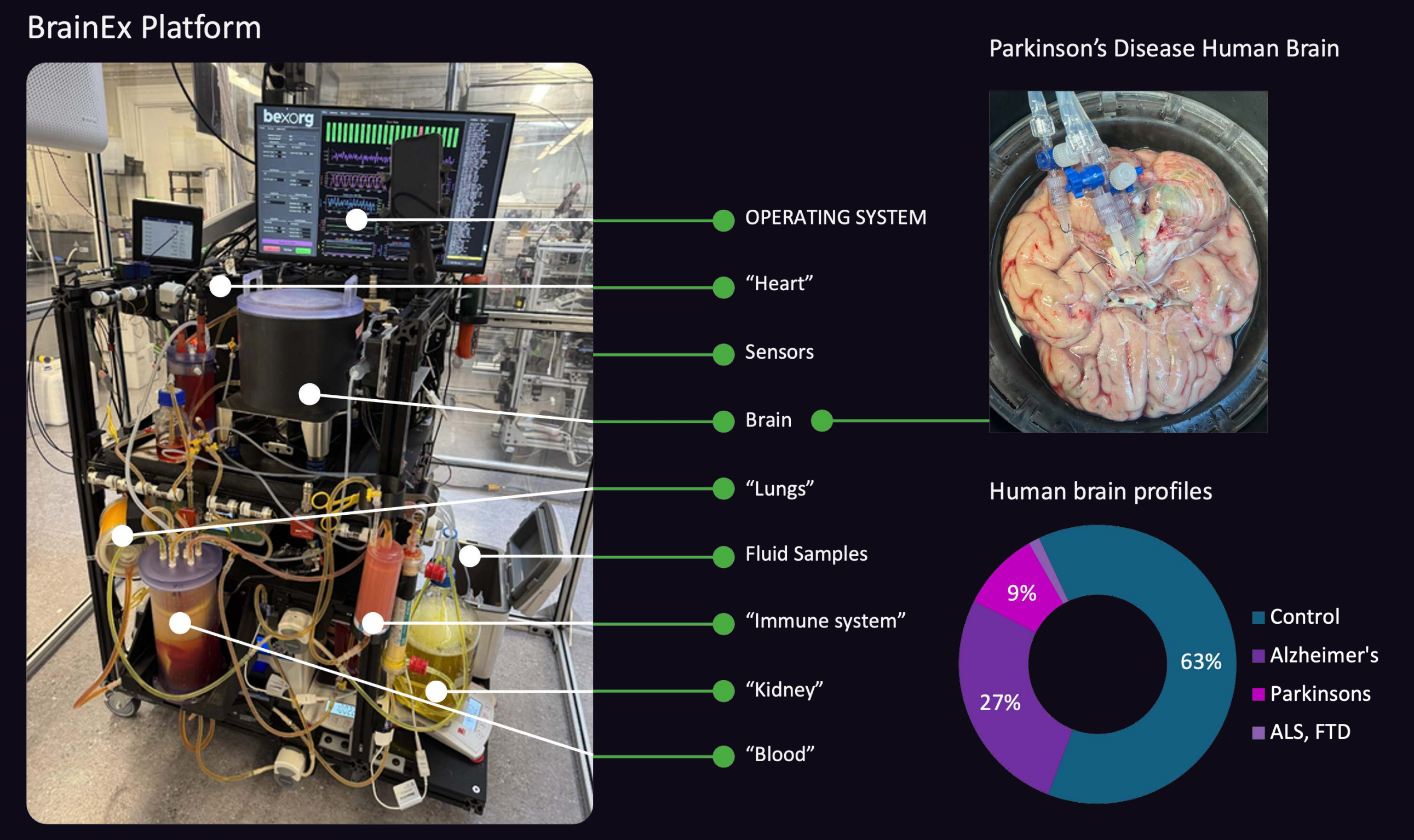


**Figure 1. The BrainEx platform enables drug discovery in whole human disease brains.** Bexorg has established a platform that maintains intact, molecularly and cellularly active postmortem human brains. The brain is connected to the BrainEx device via its endogenous vascular system. An acellular artificial perfusate supplies the brain with oxygen and nutrients, while a real-time operating system regulates physiological homeostasis. Drugs can be administered to the brain systemically, and pharmacokinetics, pharmacodynamics, and functional pharmacology can be assessed longitudinally in brain tissue and translational biofluids.

## [2] BEX-001 PROGRAM -
## RESTORING MITOCHONDRIA-LINKED BIOENERGETICS IN NEURODEGENERATION

*Metabolic pathway of interest:*

***Efficacy Biomarker A* → *Efficacy Biomarker B***
***+ATP (Energy)***

Neurodegeneration is increasingly associated with impaired bioenergetics, including mitochondrial dysfunction, reduced metabolic flexibility, and diminished ATP-generating capacity. In Alzheimer's disease and other neurodegenerative disorders, these deficits are thought to contribute directly to synaptic dysfunction, cellular stress, and progressive loss of neuronal resilience. BEX-001 is a small molecule designed to modulate a key mitochondria-linked bioenergetic pathway in human brain tissue. We hypothesized that pharmacologic engagement of this pathway would enhance metabolic activity and generate a measurable pharmacodynamic and biomarker response, with the greatest effects in neurodegeneration-affected brains.

References
Daniele, S. G., Trummer, G., Hossmann, K. A., et al. (2021). Brain vulnerability and viability after ischaemia. Nature Reviews Neuroscience, 22, 553–572. https://doi.org/10.1038/s41583-021-00488-y
Andrijevic, D., Vrselja, Z., Lysyy, T., et al. (2022). Cellular recovery after prolonged warm ischaemia of the whole body. Nature, 608, 405–412. https://doi.org/10.1038/s41586-022-05016-1
Vrselja, Z., Daniele, S. G., Silbereis, J., et al. (2019). Restoration of brain circulation and cellular functions hours post-mortem. Nature, 568, 336–343. https://doi.org/10.1038/s41586-019-1099-1

## [3] DEMOGRAPHICS

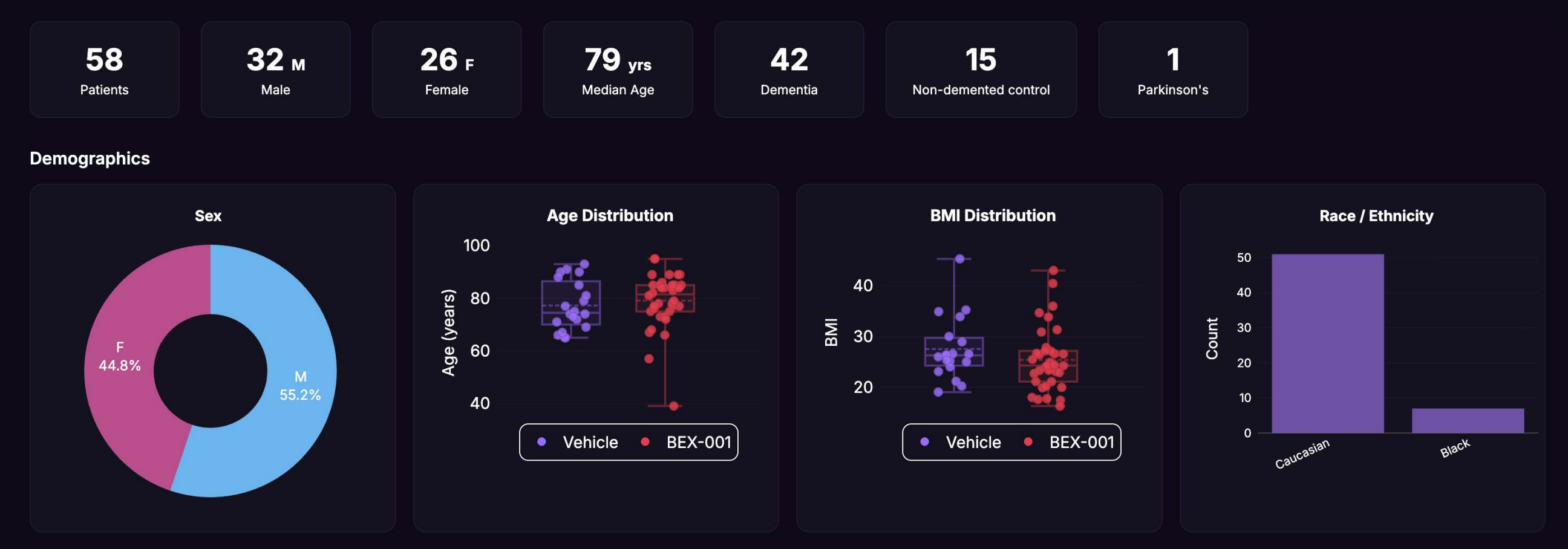


**Figure 2. Demographic characteristics of the BrainEx BEX-001 donor cohort.** Cohort summary statistics are shown above (n = 58; 32 male, 26 female; median age, 79 years). Plots show the distributions of sex, age, BMI, and race/ethnicity across donors.

## [4] PHARMACOKINETICS (PK)

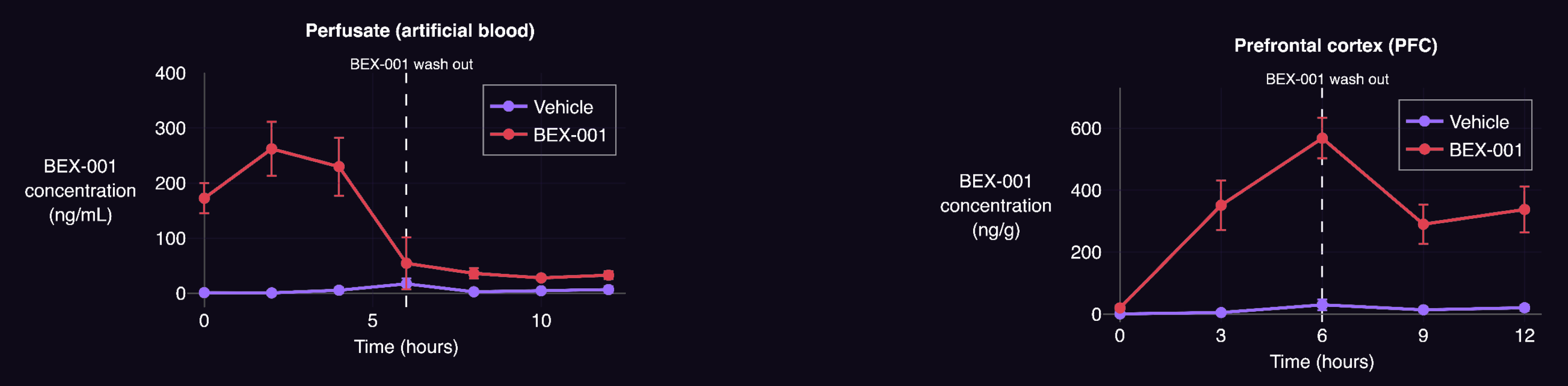


**Figure 3. Pharmacokinetics of BEX-001 on the BrainEx platform.** BEX-001 (0.6 µg/mL) or vehicle control was administered systemically via the BrainEx perfusate circuit and quantified longitudinally over 12 h by LC-MS in circulating perfusate (ng/mL) (left) and prefrontal cortex (PFC) tissue (ng/g) (right). BEX-001 administration in the perfusate resulted in full bioavailability in the circulating perfusate (left) and gradually redistributed into the brain (right). At 6 h, a washout step was initiated, clearing BEX-001 from the perfusate (left). Drug concentration in brain tissue subsequently decreased, though a substantial drug depot remained in the brain (right).

## [5] PHARMACODYNAMICS (PD)

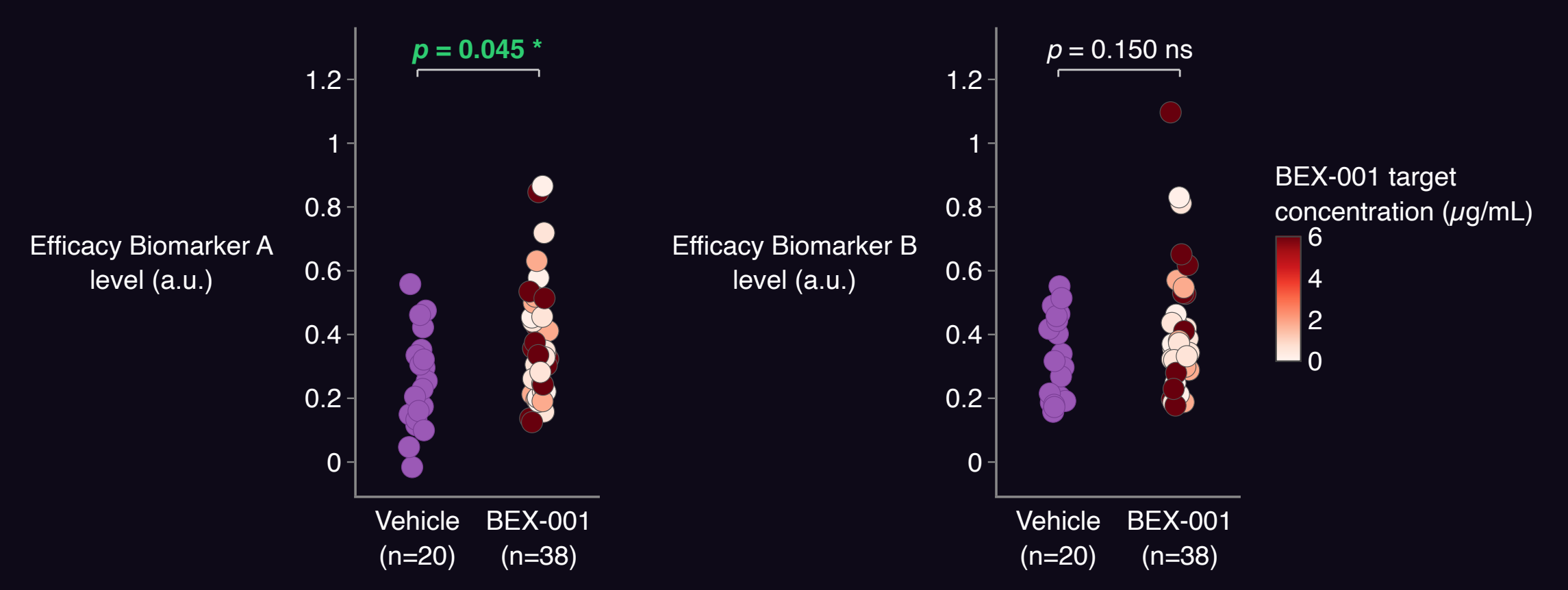


**Figure 4. Efficacy of BEX-001 in BrainEx-perfused human brains.** BEX-001 or vehicle was delivered through the BrainEx perfusate, and efficacy biomarker levels were compared across all human brains. Each point represents one perfusion run/brain (vehicle, n = 20; BEX-001, n = 38), with BEX-001 samples colored by target concentration (0 – 6 µg/mL). BEX-001 significantly increased Efficacy Biomarker A (p = 0.045) but did not alter Efficacy Biomarker B (p = 0.150, ns).

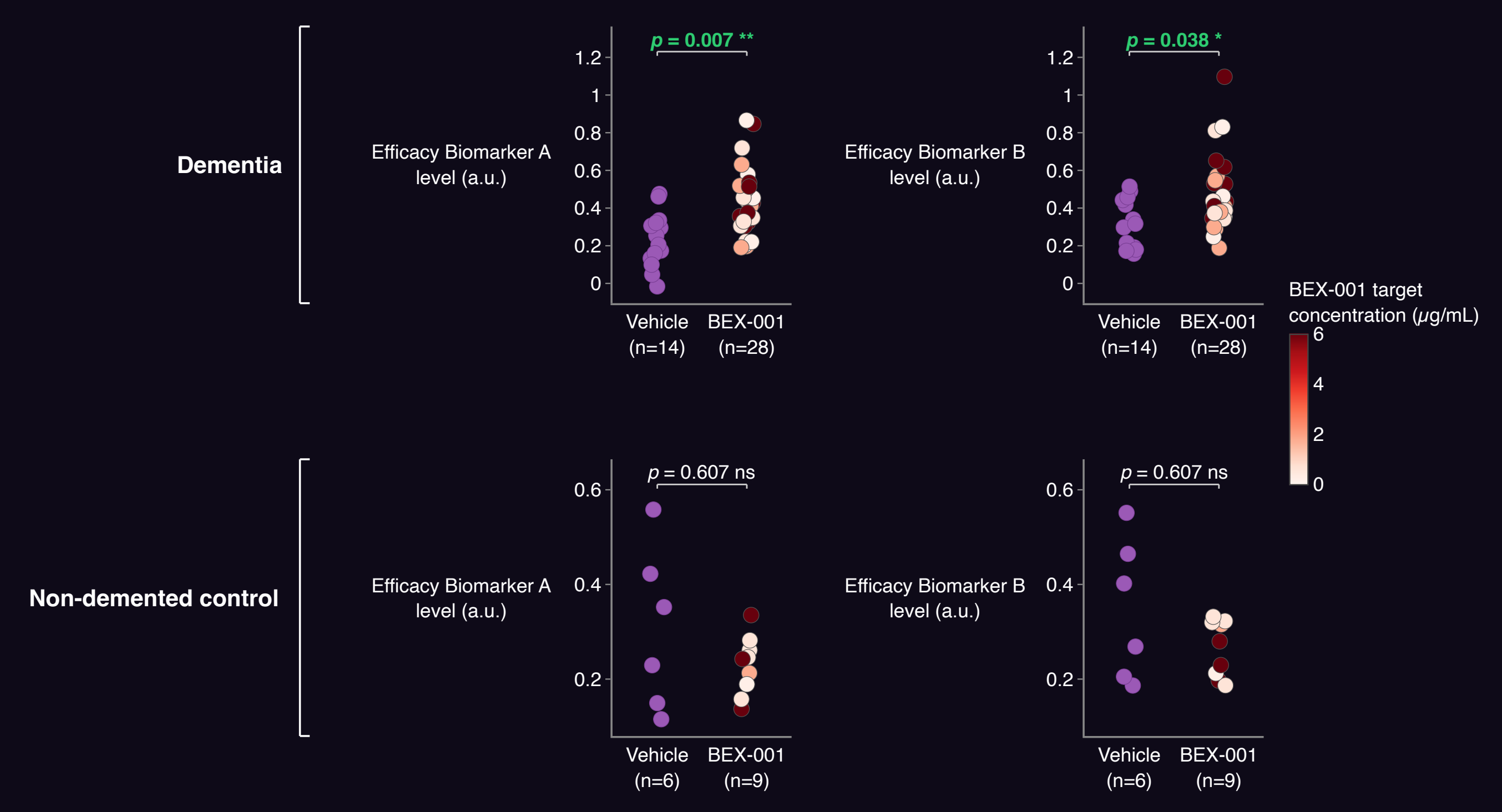


**Figure 5. Clinical diagnosis stratifies the efficacy of BEX-001 in BrainEx-perfused human brains.** In dementia brains, BEX-001 significantly increased Efficacy Biomarker A and Efficacy Biomarker B versus vehicle, whereas neither biomarker changed significantly in non-demented controls. Each point represents one perfusion run/brain, with BEX-001 samples colored by achieved target concentration (0 – 6 µg/mL).

## [6] MECHANISM OF ACTION

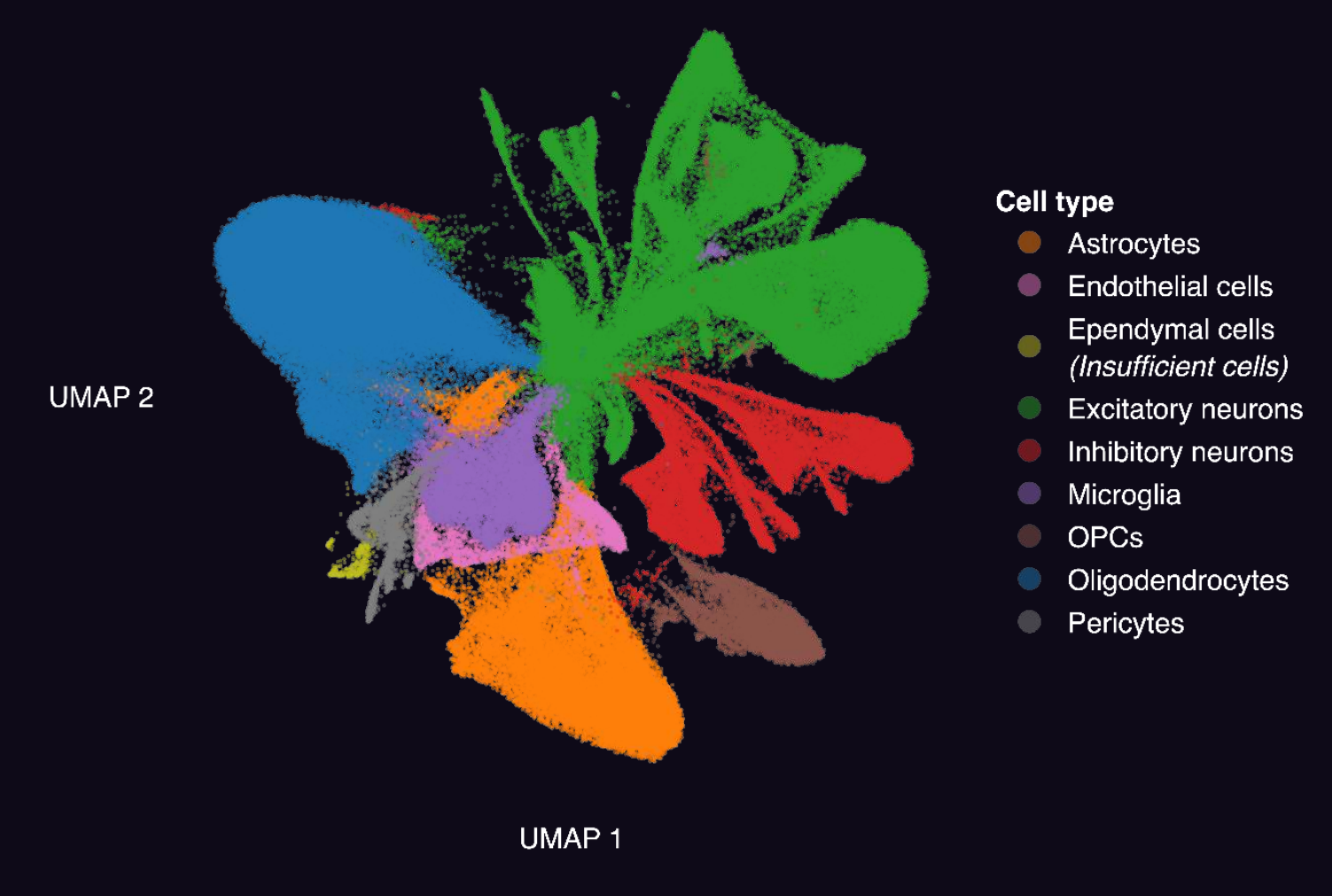
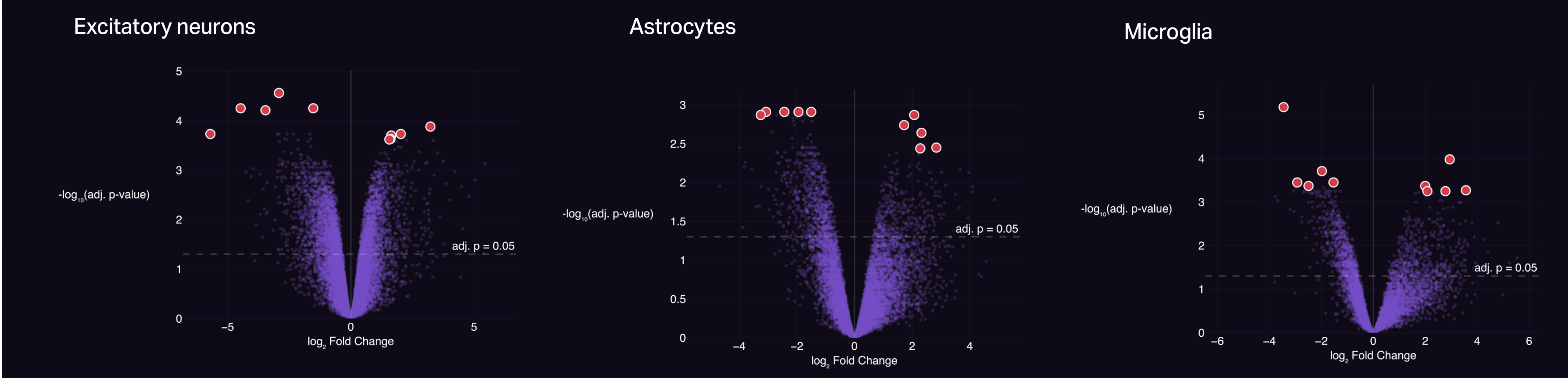


**Figure 6. Single-nucleus RNA sequencing of post-perfusion human brain tissue identifies cell-type-specific transcriptional responses to BEX-001.** Brain tissue collected after BEX-001 or vehicle perfusion was profiled by snRNA-seq. Top, UMAP of nuclei colored by annotated major brain cell types, defining the cellular landscape of the post-perfusion tissue. Bottom, cell-type-specific volcano plots comparing BEX-001- versus vehicle-treated samples in representative populations (excitatory neurons, astrocytes, and microglia), displayed as log2 fold change versus −log10 adjusted p-value. The top 10 upregulated genes in each cell type are highlighted in red.



## [7] TRANSLATIONAL BIOMARKERS

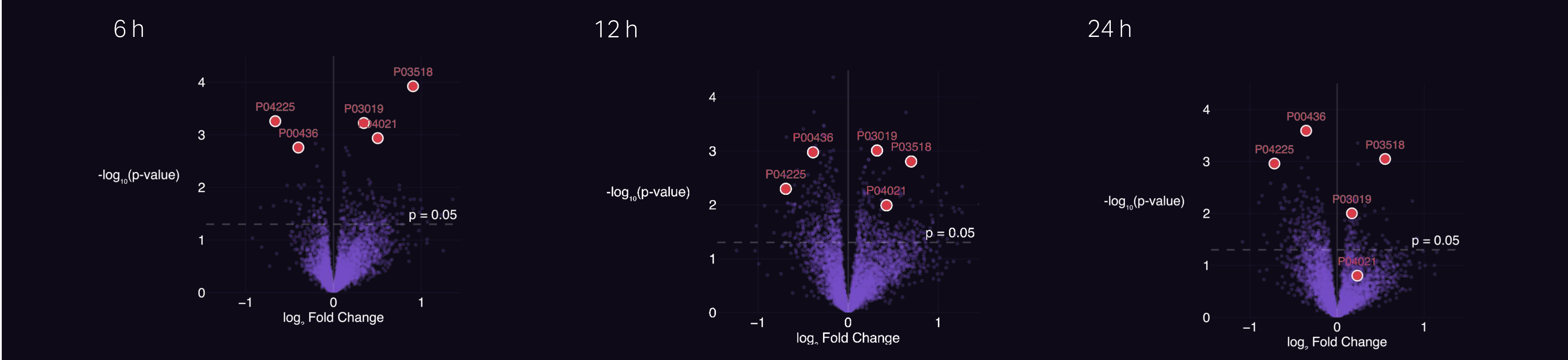


**Figure 7. Time-resolved volcano plots of perfusate proteomics after BEX-001 treatment.** Differential protein expression between BEX-001 and vehicle was assessed in BrainEx perfusate at 6, 12, and 24 h using SomaScan 11K. Each plot displays log2 fold change versus −log10(p-value), with candidate panel proteins highlighted in red and the nominal significance threshold marked at p = 0.05. The data reveal time-dependent and recurring candidate biomarkers of BEX-001 response in translational biofluids.

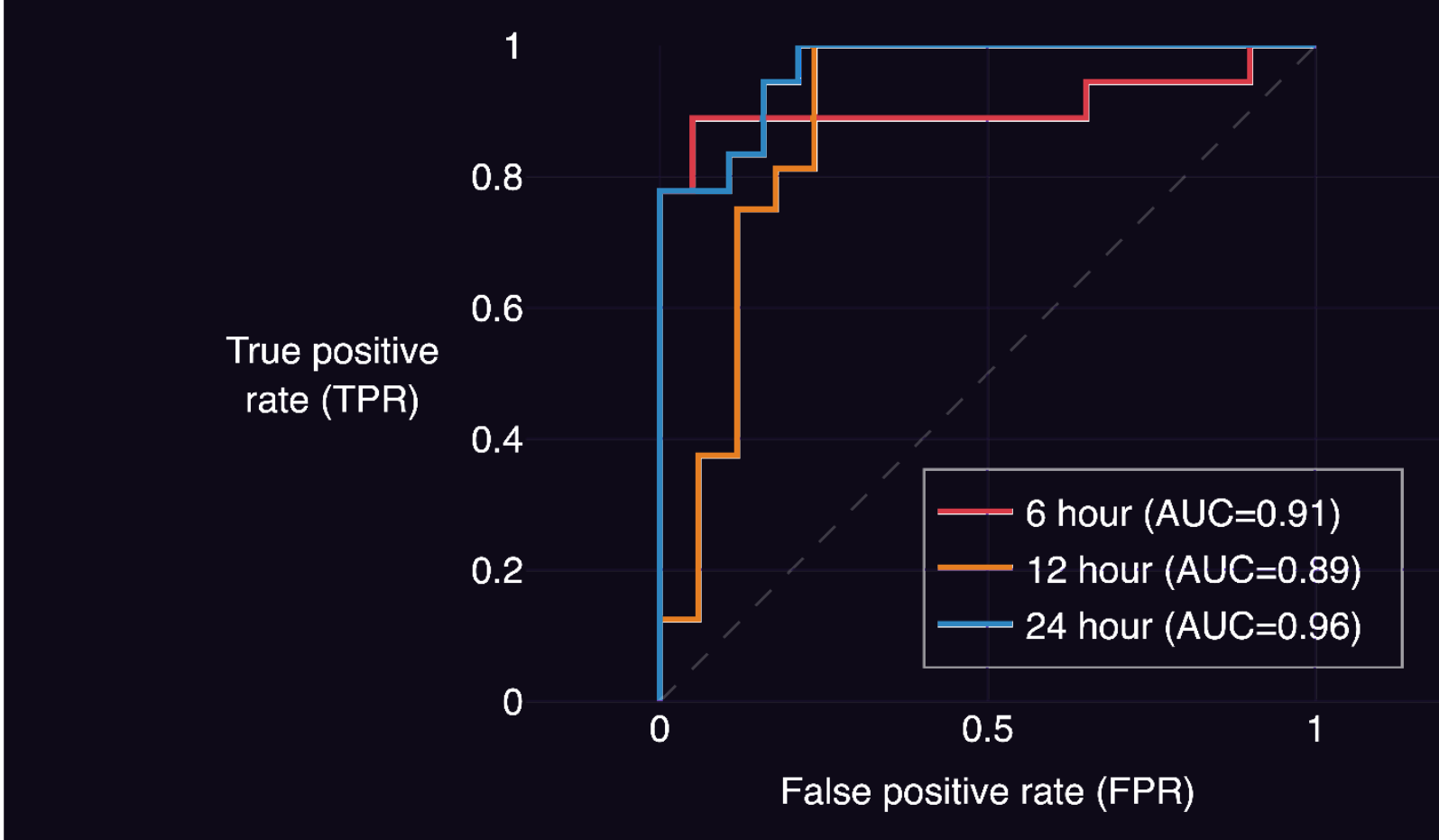


**Figure 8. Longitudinal perfusate proteomics identifies a BEX-001-responsive biomarker signature.** Machine-learning classifiers trained on proteomic profiles from translational biofluid (perfusate) samples collected at 6, 12, and 24 h during BrainEx perfusion discriminated BEX-001 from vehicle with high accuracy (AUC = 0.91, 0.89, and 0.96, respectively). The dashed diagonal line indicates chance performance (AUC = 0.5).

## SUMMARY

BrainEx enables translational CNS drug discovery directly in intact postmortem human brains by supporting longitudinal assessment of drug exposure, pharmacology, and biomarker response in native human tissue. In the BEX-001 program, systemic delivery through the perfusate achieved full bioavailability in circulation, gradual redistribution into the prefrontal cortex, and a persistent tissue depot after a 6-hour washout, demonstrating sustained brain exposure on the platform. Pharmacodynamically, BEX-001 increased Efficacy Biomarker A across all brains and, when stratified by clinical diagnosis, significantly increased both Efficacy Biomarker A and Efficacy Biomarker B in dementia brains, while no significant effects were observed in non-demented controls. Longitudinal perfusate proteomics identified a robust treatment-responsive biomarker signature, with machine-learning classifiers distinguishing BEX-001 from vehicle with high accuracy across 6, 12, and 24 hours. In parallel, single-nucleus RNA sequencing of post-perfusion tissue revealed cell-type-specific transcriptional responses to BEX-001. Together, these findings show that BrainEx can simultaneously measure PK, disease-relevant pharmacology, and translational biomarkers in the human brain, supporting BEX-001 as a promising modulator of mitochondria-linked bioenergetics in neurodegeneration.