Human-Relevant Non-Animal Methodologies (NAMs)
Specialised Models Series: **Neurological & Psychiatric**

Neuroinflammation & Glial Models

Reference 3.5 in Full Scope Roadmap

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Compiled for the Alliance for Cruelty Free Science by Georgina Rednall
Prepared for Academic Leadership, Animal Welfare & Ethical Review Bodies (AWERB), and Principal Investigators

Legal & Scientific Imperative for Universities

Section 2A of the *Animals (Scientific Procedures) Act 1986* imposes a statutory duty on the Secretary of State to ensure compliance with the principles of replacement, reduction, and refinement. The principle of replacement requires that, wherever a scientifically satisfactory method or testing strategy not entailing the use of protected animals is available, it must be used to replace the use of a regulated procedure.

Historically, neurobiology pipelines have evaluated neuroimmune signaling in transgenic mice or by harvesting primary rodent microglia and astrocytes. However, these platforms suffer from severe translational limitations, as the neuroimmune axis exhibits some of the widest evolutionary gaps between rodents and humans. These limitations are evident in the following critical disparities:

- **Gene Expression and Signalling Divergence:** Species-specific differences in microglial gene expression profiles (such as human-specific activation states of Disease-Associated Microglia) and toll-like receptor (TLR) signaling pathways render rodent models non-predictive of human clinical responses.

- **Astrocyte Morphological and Functional Complexity:** Human astrocytes are significantly larger, possess up to tenfold more processes, and exhibit entirely distinct reactive phenotypes—such as the neurotoxic A1/A2 astrocyte dichotomy—compared to their rodent counterparts.

- **Demyelination and Differentiation Mismatch:** In demyelinating diseases like Multiple Sclerosis (MS), species-specific differences in oligodendrocyte progenitor cell (OPC) differentiation kinetics and myelination patterns render rodent autoimmune encephalomyelitis (EAE) assays highly unreliable.

Accelerating the transition to human-relevant Non-Animal Methodologies (NAMs) is an urgent scientific necessity to bridge the translation gap and a statutory mandate for UK academic research programs under *ASPA 1986*.

Landmark Regulatory & Scientific Momentum

Significant progress has been made in the development and application of human-relevant neuroinflammatory and glial NAMs:

- **Neuroimmune-Competent Brain-on-Chip Models:** Incorporate functional, patient-derived microglia, astrocytes, and neurons under continuous microfluidic shear stress to model the complex cellular crosstalk of the neuroinflammatory cascade.
- **Myelination-on-Chip Platforms:** Utilise engineered human primary or iPSC-derived oligodendrocytes co-cultured with aligned axonal matrices to track sheath assembly and demyelinating injury in real time.
- **3D Spatial Neuroinflammatory Organoids:** Leverage advanced spatial biology and transcriptomics to map localised glial reactive phenotypes, microglial migration, and pro-inflammatory cytokine secretomes.

Core Specialised Neuroinflammatory & Glial Platforms

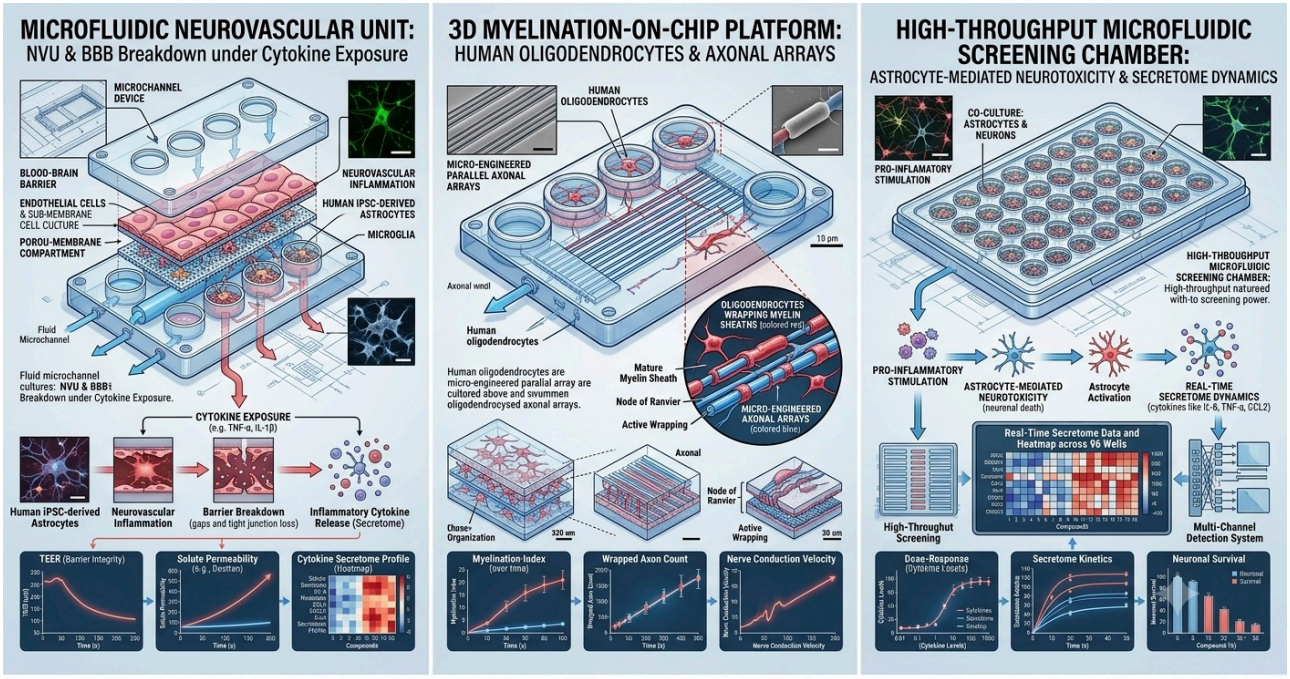

Figure 1: Core Specialised Neuroinflammatory & Glial Platforms — Microfluidic neurovascular unit incorporating human iPSC-derived microglia and astrocytes to capture neurovascular inflammation and blood-brain barrier breakdown under cytokine exposure *(left)*; 3D myelination-on-chip platform demonstrating human oligodendrocytes wrapping myelin sheaths around micro-engineered axonal arrays *(centre)*; and a high-throughput microfluidic screening chamber tracking astrocyte-mediated neurotoxicity and real-time pro-inflammatory biomarker secretome dynamics *(right)*.

Table 1: Specialised Biological & Tissue-Engineered (In Vitro) Platforms

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
Neuroimmune-Competent Microfluidic Chips	Dynamic microfluidic chambers co-culturing human primary or iPSC-derived neurons, astrocytes, and active microglia under shear stress.	Modeling microglial migration, blood-brain barrier neurovascular compromise, and localized pro-inflammatory cytokine profiling.	Replaces high-stress rodent systemic LPS injection models and subsequent terminal brain tissue harvesting.
Myelination & Demyelination-on-Chip	Engineered micro-channels co-culturing human oligodendrocytes with long-projection human axonal scaffolds.	Real-time tracking of myelin sheath assembly, oligodendrocyte progenitor cell (OPC) maturation, and demyelinating plaques in Multiple Sclerosis.	Directly replaces the highly painful rodent Experimental Autoimmune Encephalomyelitis (EAE) model of paralysis.
3D Synovial & Astrocyte Reactive Organoids	Self-organizing brain organoids integrated with patient-derived reactive astrocytes and microglia.	Profiling disease-associated microglial (DAM) states, tracking astrocyte-induced neuronal cell death, and modeling chronic neuroinflammation.	Replaces rodent stereotaxic chemical brain-lesion models and terminal immunohistochemistry evaluations.
Microfluidic Glial-Lymphatic (Glymphatic) Units	Microvascular channels lined with human endothelial cells co-cultured with astrocyte end-feet expressing functional aquaporin-4 (AQP4).	Investigating glymphatic clearance mechanics, waste transport kinetics, and neuroinflammatory swelling dynamics.	Replaces highly invasive, live-animal cranial window imaging and cerebrospinal fluid tracer infusion assays.

Cross-Cutting & Enabling Platforms for Neuroinflammatory Research

These platforms support multiple disease-specific research areas and are widely applicable across small-molecule design, genetic target validation, and pharmacokinetic profiling. While tissue-specific organoids focus on structural niches, these cross-cutting technologies provide the quantitative pipelines required to validate high-maturity NAMs for regulatory-accepted, "fit-for-purpose" research.

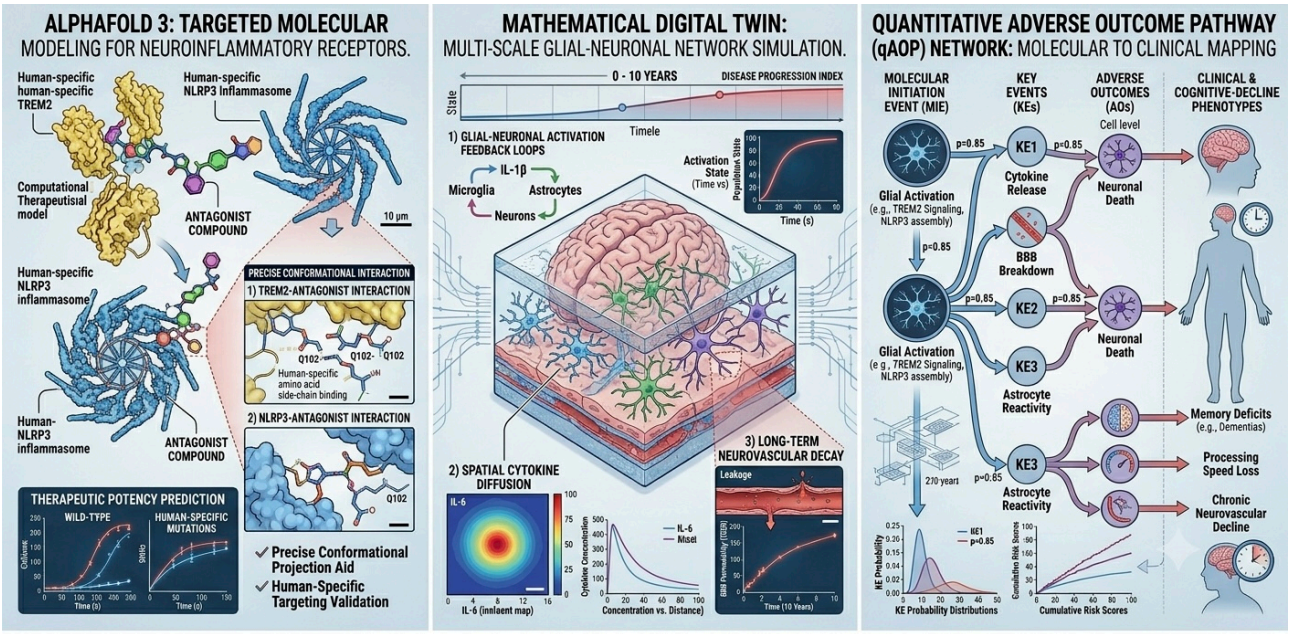

Figure 2: Cross-Cutting & Enabling Platforms for Neuroinflammatory Research — AlphaFold 3 molecular modeling projecting the precise conformational interaction of therapeutic antagonists with human-specific neuroinflammatory receptor targets (such as TREM2 and the NLRP3 inflammasome) *(left)*; mathematical digital twin simulating multi-scale glial-neuronal activation feedback loops, spatial cytokine diffusion, and long-term neurovascular decay *(centre)*; and a quantitative Adverse Outcome Pathway (qAOP) network mapping molecular initiation events (e.g., glial activation) to clinical, cognitive-decline phenotypes *(right)*.

Table 2: Genetic, Computational & Hybrid Platforms

Platform Type	Technology Application	Impact on 3Rs/Replacement
Genetic/ Molecular	CRISPR/Cas9-engineered human iPSC-microglia	Replaces primary rodent microglia harvesting
Computational	AI-driven simulation of human-specific TLR signaling	Bypasses non-predictive murine neuroimmune assays
Hybrid (MPS)	Microfluidic "Brain-on-a-Chip" (Glial-inclusive)	Eliminates rodent EAE and demyelination models

Regulatory Software & Strategy Frameworks

Regulatory acceptance of NAMs—referred to as "fit-for-purpose qualification"—is not a one-size-fits-all process. Researchers should utilise the following frameworks to ensure their computational and biological strategies align with the *Animals (Scientific Procedures) Act 1986* and industry best practices:

- **Context of Use (COU) Documentation:** Before deploying models, clearly define the specific scientific question the model is intended to address. This is critical for demonstrating that the NAM is reliable and suitable for its intended regulatory purpose.
- **Technical Characterisation:** Maintain comprehensive Standard Operating Procedures (SOPs) and study plans for both the biological NAM and any software used to analyse its output. This transparency is vital for successful project licence applications.
- **Early Regulatory Engagement:** Leverage institutional or external regulatory support to track "fit-for-purpose" milestones. Early engagement with the Home Office/ASRU is strongly encouraged to test the acceptability of a computational or NAM-based approach before full project submission.
- **Weight-of-Evidence (WoE) Frameworks:** Use integrated digital dashboards to aggregate data from both *in vitro* (NAM) and *in silico* (computational) sources. Building a strong "weight-of-evidence" argument is the most effective way to strengthen Replacement justifications when moving away from traditional animal models.

Skills, Training, and Capacity Building

Successful adoption of high-maturity Non-Animal Methodologies (NAMs) requires a strategic shift in workforce development. To ensure the long-term success of the platforms outlined in this series, universities should prioritise the following training initiatives to build institutional capacity:

- **Integrated Neurobiology & Microengineering:** Training programmes should bridge the gap between bench-top cellular biology, fluidic microengineering, and complex electrophysiology data analysis. Researchers require dual proficiency in maintaining long-term fluidic circuit barriers and leveraging the computational pipelines necessary to interpret high-density microelectrode array data.
- **Regulatory Competency:** Staff should receive formal training on "Fit-for-Purpose" qualification and the specific regulatory expectations of the Home Office/ASRU. This includes understanding how to document evidence for the 3Rs within formal project licence applications to ensure they meet legal standards.
- **Technological Literacy:** Skills in microfluidic device troubleshooting, primary stem-cell tissue differentiation, brain organoid maintenance, and AI-driven structural computational modelling are increasingly critical for modern neurobiology research. Universities should facilitate internal workshops or establish external partnerships to provide PhD students and postdoctoral fellows with hands-on experience in these specialised tools.
- **Ethics & Translation:** Education should focus on the scientific imperative of human-relevant research, emphasising why NAMs provide more predictive insights for clinical outcomes than traditional rodent models. This fosters a culture of excellence and ethical research design that is fully aligned with modern international standards.

Table 3: Skills • Training & Capacity Building

Capability Domain	Focus Area	Impact on 3Rs/Replacement
Technical	Advanced human iPSC-microglia & astrocyte culture	Eliminates harvesting from primary rodent brain tissue
Computational	AI-modelling of human-specific TLR signaling	Bypasses non-predictive murine neuroimmune assays
Regulatory	Fit-for-Purpose validation of glial-inclusive MPS	Ensures ASPA 1986 compliance for neuro-models
Bioinformatics	Transcriptomic analysis of Disease-Associated Microglia	Replaces animal-based inflammatory pathway studies

Strategic Note: This investment in human expertise is a key component of a robust, future-proof institutional roadmap, demonstrating to regulators that the institution is committed to the safe and effective operation of high-maturity NAMs.

Clinical Translation Case Studies

Case Study 1: Human Neurovascular Unit-on-Chip Correcting Rodent Anti-Inflammatory Target Failures (MS)

- **Background:** A highly promising therapeutic candidate targeting a novel microglial receptor for demyelinating lesions in Multiple Sclerosis demonstrated excellent myelin repair rates in rodent models of EAE. However, during early human clinical trials, the candidate failed completely because the target receptor exhibited significantly different expression profiles and downstream activation cascades in human microglia.

- **The Paradigm Shift:** An academic research group re-evaluated the failed candidate using a neuroimmune-competent human Neurovascular Unit (NVU)-on-chip model. The human-relevant platform correctly predicted the clinical failure, demonstrating that the mouse-derived neuroimmune activation kinetics were non-equivalent to human cells.

- **The Methodology:** Researchers generated microfluidic chips containing human iPSC-derived brain microvascular endothelial cells, astrocytes, and patient-derived microglia. They applied the therapeutic lead and quantified real-time microglial receptor occupancy, phagocytic clearing of myelin debris, and neurovascular barrier integrity.

University & Legal Takeaway: This study highlights how high-fidelity human neurovascular chips can successfully replace rodent EAE efficacy screening, providing solid legal justification to the Home Office under ASPA 1986 that animal testing was non-predictive.

Case Study 2: Human Myelination-on-Chip Replacing High-Severity Animal EAE Models (MS)

- **Background:** Traditional pre-clinical evaluation of myelin regeneration and neuroprotective drugs historically relies on inducing Experimental Autoimmune Encephalomyelitis (EAE) in rodents. These animals suffer severe, progressive ascending paralysis, hind-limb loss, and immense systemic distress before being sacrificed. This high-severity model has a poor track record of predicting clinical efficacy in human patients.

- **The Paradigm Shift:** A university research team implemented a high-throughput, human-relevant Myelination-on-Chip platform, completely replacing the need for high-severity rodent EAE models for target validation.

- **The Methodology:** The team co-cultured human iPSC-derived oligodendrocytes with aligned human sensory axons on compartmentalised microfluidic substrates. They validated the therapeutic lead by measuring oligodendrocyte progenitor cell (OPC) recruitment, real-time myelin sheath wrapping, and electrical conduction velocity recovery under cytokine challenge.

- **University & Legal Takeaway:** Demonstrates a highly predictive human alternative that completely replaces high-severity animal protocols, aligning perfectly with Home Office expectations for the application of high-maturity Replacement models.

Executive Summary & Compliance Dashboard

Executive Summary

Neuroinflammation and glial cell dysregulation are critical pathological drivers across the spectrum of neurological, psychiatric, and neurodegenerative conditions. Historically, research in this domain has relied on primary rodent cells or transgenic mice; however, these models suffer from significant evolutionary gaps in microglial gene expression, astrocyte morphology, and toll-like receptor (TLR) signaling pathways. This roadmap mandates a transition to high-fidelity, human-relevant NAMs—specifically human iPSC-derived glial cultures and microfluidic "Brain-on-a-Chip" platforms—to replace non-predictive animal assays and align institutional research with the Section 2A statutory duty of *ASPA 1986*.

Compliance Dashboard

Focus Area	Primary Animal Model	NAM Replacement Technology	ASPA 1986 Status
Neuroinflammation	Rodent EAE & Primary Glial Harvesting	iPSC-Glial Organoids & Glial-MPS	High

See Appendix: Animal-Free Matrix for the long term strategic implementation roadmap

Maturity Matrix

Phase 1 (Exploratory): Audit current reliance on primary rodent glial harvesting and EAE models.

Phase 2 (Pilot): Integrate human iPSC-derived microglia and astrocyte cultures into neuro-pipelines.

Phase 3 (Integration): Deploy glial-inclusive microfluidic systems to model multi-lineage neuroimmune interactions.

Phase 4 (Standardisation): Replace 100% of non-predictive murine neuroimmune and demyelination assays with human-relevant NAMs.

Final Compliance Statement

All proposed models satisfy the Section 2A statutory duty of *ASPA 1986*. By adopting these methodologies, the institution positions itself at the forefront of the global transition toward human-centric, cruelty-free medical science.

Integration Context: Roadmap Conclusion

The modules detailed in this roadmap—from the fundamental neuroinflammatory models explored in this series to the broader psychiatric and neurodegenerative frameworks established previously—demonstrate that replacement is an integrated institutional strategy. By mapping these specialised NAM-based glial platforms into a unified regulatory framework, the institution moves decisively toward a standardised, human-centric research paradigm. This systemic integration is the essential prerequisite for fulfilling the *ASPA 1986* statutory duty, ensuring all research is scientifically accurate, ethically aligned, and regulatory compliant.

Appendix: Animal-Free Maturity Matrix

Neuroinflammation & Glial Models Research Roadmap

This matrix maps the transition from legacy transgenic rodent models and primary glial harvesting to human-relevant, animal-free methodologies for neuroinflammatory diseases like Multiple Sclerosis (MS), in compliance with the Animals (Scientific Procedures) Act 1986 (ASPA 1986).

Technology Platform	Phase 1: Exploration	Phase 2: Integration	Phase 3: Validation	Phase 4: Adoption
Glial MPS Chips	Microfluidic "Brain-on-Chip" design.	Neuroimmune-competent co-culture.	Integration into WoE frameworks.	High-throughput inflammatory profiling.
iPSC Organoids	Self-organising 3D organoid formation.	Glial integration (astrocyte/microglia).	Multi-lab validation; trait modelling.	Regulatory-standardised use.
In Silico Twins	Computational network design.	AI-driven inflammatory signaling modeling.	Predictive longitudinal modeling.	Primary tool for target discovery.
Hybrid (MPS) Systems	Kinetic simulation of metabolic waste.	Human-specific TLR signaling simulations.	Data benchmarking vs. legacy models.	Core standard for efficacy testing.

Implementation & Compliance Framework

To ensure institutional adherence to the **Animals (Scientific Procedures) Act 1986 (ASPA 1986)**, the following five-phase roadmap must be applied to all neuroinflammatory research projects:

Phase 1: Gap Analysis & Resource Audit
Audit current protocols to identify legacy rodent EAE and demyelination models suitable for transition to NAMs.

Assess institutional capacity for iPSC-derived technologies and high-performance computing.

Phase 2: Standardised SOP Development
Develop rigorous SOPs for human-specific TLR signalling simulations and glial-inclusive MPS chips.

Replace animal-based inflammatory pathway studies and primary rodent brain tissue harvesting with standardised human iPSC-derived protocols.

Phase 3: Weight-of-Evidence (WoE) Dossier Construction
Aggregate performance data from multi-organ simulations, including transcriptomic analysis of Disease-Associated Microglia (DAM).

Benchmark NAM outputs against historical legacy data to prove "fit-for-purpose" status for human-specific inflammatory modelling.

Phase 4: Regulatory Alignment & Dashboard Tracking
Utilise a compliance dashboard to report project milestones directly to the ASRU.

Leverage detailed WoE documentation to justify the total cessation of rodent EAE models.

Phase 5: Continuous Capability Building
Invest in specialised staff training for digital twin and synaptic chip operation to bridge the gap between bench-top and computational neuroimmunology.

Strategic Note: This investment in human expertise is a key component of a robust, future-proof institutional roadmap, demonstrating to regulators that the institution is committed to the safe and effective operation of high-maturity NAMs.