**Human-Relevant Non-Animal Methodologies (NAMs)**
*Specialised Models Series:* **Immune & Infectious Disease**

# Autoimmune Disease Models

**Reference 2.3 in Full Scope Roadmap**

Version 1.0 | June 2026 Edition
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## 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.

Traditional animal models in cancer immunotherapy research—particularly murine xenograft and syngeneic models—have significant limitations due to species differences in immune surveillance, tumour microenvironment (TME) architecture, and checkpoint signalling. These limitations are evident in the following critical disparities:

- **TME Architecture & Immune Exclusion:** Human tumours exhibit complex, patient-specific TME structures that govern immune cell infiltration. Rodent models frequently fail to recapitulate the physical and biochemical barriers to immune-mediated tumour killing, leading to poor predictive value for clinical efficacy.

- **Checkpoint & Receptor Species-Specificity:** Human-specific checkpoint targets (e.g., PD-1/PD-L1, CTLA-4) and co-stimulatory molecules often demonstrate limited functional cross-reactivity with rodent counterparts. This necessitates the use of complex "humanised" mice, which are costly, prone to variability, and still fail to replicate the full breadth of human immune-tumour interaction.

- **Tumour Heterogeneity & Evolution:** The clonal evolution and genetic heterogeneity characteristic of human cancers are rarely captured in standard animal models. Consequently, the rapid emergence of therapeutic resistance observed in human patients is often masked or misrepresented in animal studies.

- **Severity of Therapeutic Evaluation:** Advanced immunotherapies (e.g., CAR-T, bispecific antibodies) frequently induce systemic inflammatory responses or cytokine release syndromes in humans that are not adequately predicted by rodent models, while simultaneously inflicting high-severity systemic stress and morbidity on the animals themselves.

Accelerating the adoption of human-relevant Non-Animal Methodologies (NAMs)—such as patient-derived tumour organoids (PDTOs), immune-tumour microfluidic chips, tumour-on-a-chip platforms, and multi-cellular immune-tumour assembloids—is therefore both a scientific imperative and a legal imperative for UK universities.

## Landmark Regulatory & Scientific Momentum

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

- **Barrier-on-Chip & Epithelial-Immune Interfaces:** Faithfully recapitulate localised tissue breakdown and autoantibody infiltration across human gut, blood-brain, or synovial membranes.
- **Patient-Specific iPSC Autoimmune Platforms:** Differentiate patient-derived stem cells into specific target tissues and autologous immune cells to model complex, multi-lineage genetic liabilities.
- **In Silico Immune Profiling & Digital Twins:** Leverage computational modelling and multi-omics data integration to forecast chronic inflammatory flares and simulate patient-specific treatment responses.

## Core Specialised Autoimmune Models & Enabling Platforms

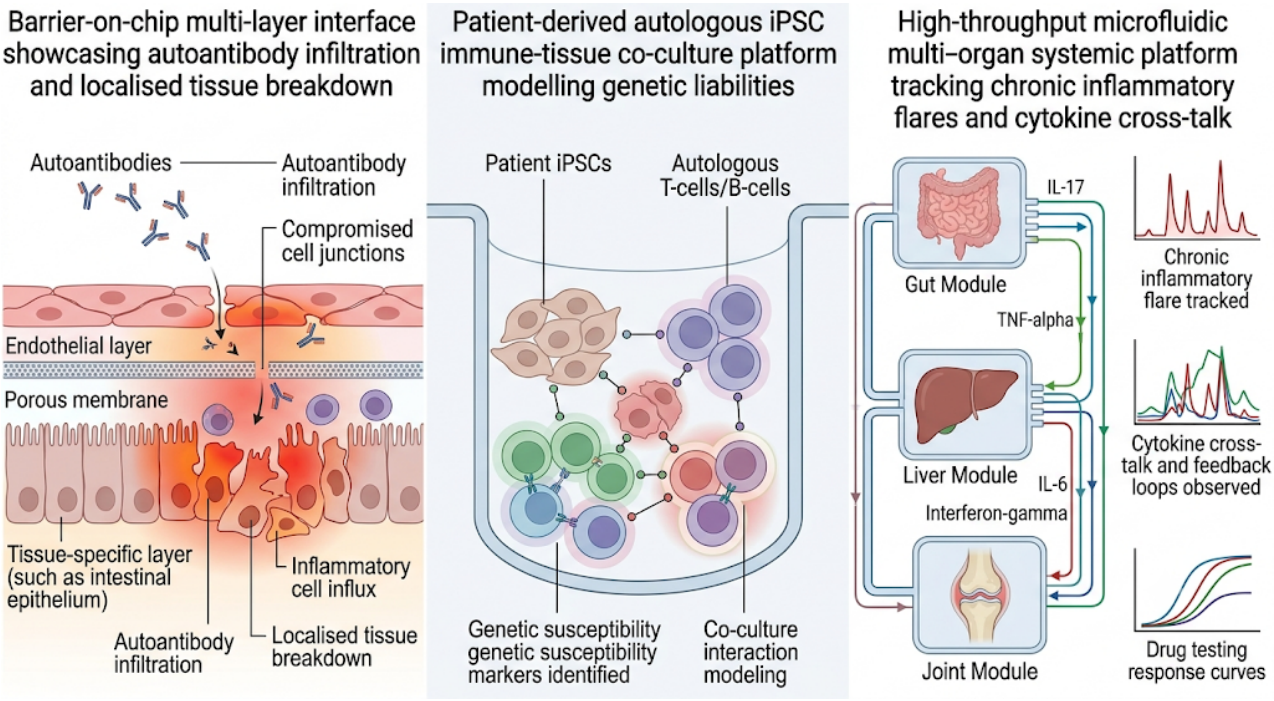


**Figure 1:** Core Specialised Autoimmune Disease Models — Barrier-on-chip multi-layer interface showcasing autoantibody infiltration and localised tissue breakdown (left), patient-derived autologous iPSC immune-tissue co-culture platform modelling genetic liabilities (centre), and high-throughput microfluidic multi-organ systemic platform tracking chronic inflammatory flares and cytokine cross-talk (right).

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

| Platform / Model | Description | Key Applications | Benefits for Universities & 3Rs |
|---|---|---|---|
| **Barrier-on-Chip & Epithelial-Immune Interfaces** | Microfluidic multi-layer polymer platforms that recreate endothelial, epithelial, or synovial membranes co-cultured with primary human immune cells under physiological fluid flow. | Synovial inflammation (RA), blood-brain barrier breakdown (MS), intestinal mucosal leakage (IBD), and autoantibody trafficking kinetics. | High human relevance for tracking localised tissue breakdown; provides alternatives to invasive animal tissue harvesting. |
| **Patient-Specific iPSC Autoimmune Platforms** | Differentiated human tissue cell clusters derived from patient stem cells, paired with autologous multi-lineage immune cells to model personalised genetic backgrounds. | Target tissue destruction assays, human leukocyte antigen (HLA) presentation tracking, and screening for patient-specific therapeutic responses. | Enables functional modeling of complex, multi-lineage human genetic liabilities without animal genetic modification. |

## Cross-Cutting & Enabling Platforms for Autoimmune Research

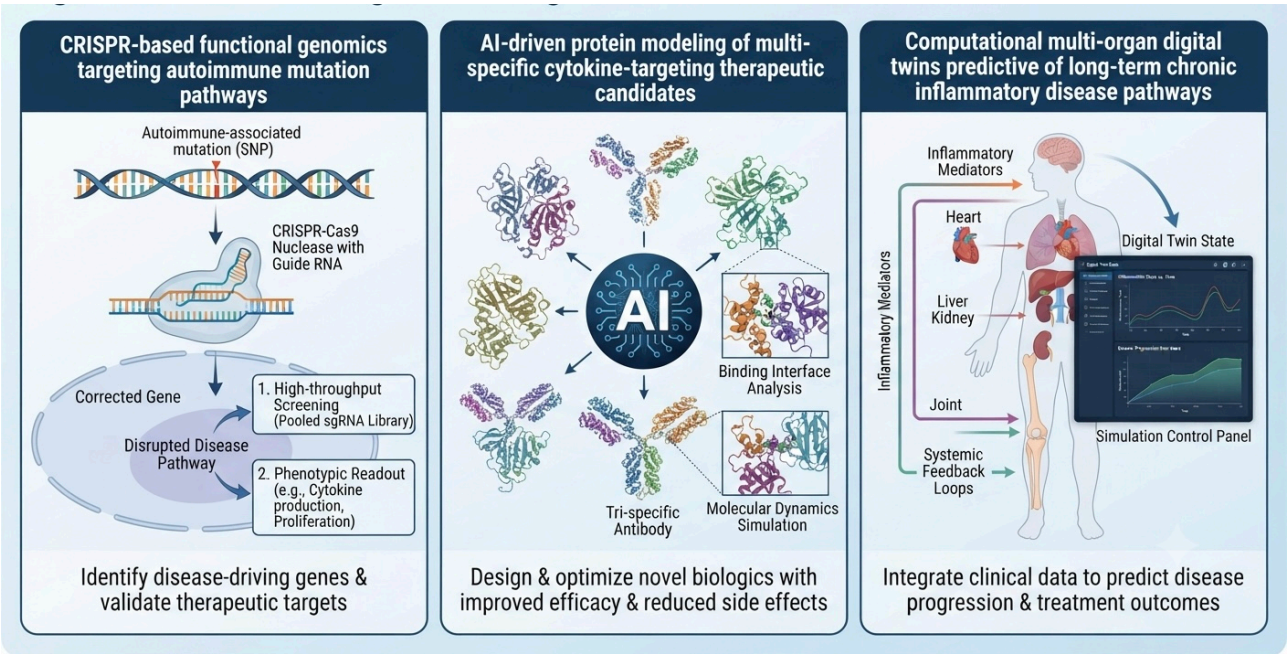


**Figure 2:** Cross-Cutting & Enabling Platforms for Autoimmune Research — CRISPR-based functional genomics targeting autoimmune mutation pathways (left), AI-driven protein modeling of multi-specific cytokine-targeting therapeutic candidates (center), and computational multi-organ digital twins predictive of long-term chronic inflammatory disease pathways (right).

## Table 2: Genetic, Computational & Framework-Driven Platforms

| Platform / Model | Description | Key Applications | Benefits for Universities & 3Rs |
|---|---|---|---|
| **CRISPR-Based Pathway & Target Validation** | Advanced gene-editing tools integrated into human iPSC lines or organoids to knock out or knock in specific susceptibility loci or cytokine receptors. | Mechanistic studies of HLA-associated liabilities, regulatory network mapping, and validation of novel intracellular therapeutic targets. | Directly circumvents species-specific gene signaling differences; provides bulletproof evidence for human genetic replacement arguments. |
| **Multi-Organ MPS & Computational Digital Twins** | Interconnected microphysiological networks (e.g., gut-immune-joint loops) paired with multi-omics data streams and predictive computational modeling software. | Tracking systemic cytokine cascades (TNF − α, IL − 17, IL − 23), forecasting long-term inflammatory flares, and multi-organ toxicity profiling. | Holistic systems biology approach; ideal for predictive translation and training researchers in advanced in silico replacement metrics. |
| **AOP-Integrated NAMs for Chronic Inflammation** | Adverse Outcome Pathway (AOP) frameworks combining in vitro multi-cell data with computational analysis to track early cellular triggers to organ-level failure. | Safety assessment, systemic immunotoxicity screening, and regulatory weight-of-evidence dossier compilation for investigational new drugs (IND). | Aligns perfectly with Home Office / ASRU standards for regulatory acceptance and "fit-for-purpose" qualification protocols. |

## 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

- **Integrated Autoimmunity & Data Science:** Training programmes should bridge the gap between bench-top cellular biology and complex multi-omics data analysis. Researchers require dual proficiency in maintaining long-term barrier-on-chip models and leveraging the computational pipelines necessary to interpret high-throughput cytokine and transcriptomic profiles.
- **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 CRISPR-based target validation, patient-derived organoid handling, and AI-driven structural modelling are increasingly critical for modern autoimmune 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, and Capacity Building

| Capability Domain | Focus Area | Impact on 3Rs/Replacement |
|---|---|---|
| **Technical** | iPSC-derived hepatocyte 3D culture | Direct Replacement of animal models |
| **Bioinformatics** | Hepatic transcriptome data analysis | Bypasses murine liver-injury histology |
| **Regulatory** | ASPA 1986 Compliance & AWERB | Facilitates faster ASRU approvals |
| **Computational** | PBPK liver-clearance simulations | Eliminates invasive animal PK 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: Patient-Derived Synovium-on-Chip Reaching Clinical Trials

- **Background:** Traditional development of rheumatoid arthritis (RA) drugs relies heavily on collagen-induced arthritis (CIA) rodent models, which fail to replicate human synovial fibroblast behaviour and localised autoantibody destruction.
- **The Paradigm Shift:** Utilising patient-derived synovial cells and autologous T-cells inside a microfluidic chip has accelerated the path to the clinic. Multiple targeted kinase inhibitors, validated using these human synovial-on-chip models, have progressed into active clinical trials.
- **The Methodology:** Researchers use microfluidic channels to subject human RA synovial constructs to physiological fluid shear stress while tracking T-cell infiltration, cytokine release (TNF-α, IL-6), and subsequent cartilage degradation. This human-relevant data supports IND submissions and refines dosing profiles before human trials.
- **University & Legal Takeaway:** These approaches provide strong non-animal evidence to support Project Licence applications, demonstrating robust replacement of animal models under the 3Rs and ASPA 1986 requirements.

### Case Study 2: AI-Driven Cytokine Modelling Entering First-in-Human Trials

AI-driven protein structure and cellular pathway modelling tools are now directly contributing to clinical candidates for autoimmune conditions. Computational platforms have advanced structural designs for multi-specific antibodies targeting complex cytokine networks simultaneously (e.g., co-inhibiting IL-17 and IL-23 pathways in psoriasis). These candidates were optimised using structural insights that would have taken years to obtain experimentally via animal serum testing, significantly shortening the timeline to early-phase clinical trials.

### Case Study 3: Multi-Organ Barrier MPS and Digital Twins in Inflammatory Bowel Disease (IBD) Trials

Pharmaceutical and biotech companies are increasingly incorporating multi-organ gut-immune microphysiological systems (MPS) alongside digital twins to model systemic immune responses, predict personalised treatment outcomes, and support clinical trial design. Digital twin technologies are already being used in ongoing trials for IBD and Crohn's disease to safely reduce patient cohorts, enhance statistical power, and enable precision dosing by accurately predicting individual mucosal healing rates.

## Executive Summary & Compliance Dashboard

### Executive Summary

Traditional animal models in metabolic and hepatic research, such as diet-induced obesity (DIO) murine models, frequently fail to recapitulate human-specific lipid metabolism and hepatotoxicity profiles, leading to significant translational gaps. This roadmap outlines a transition to high-fidelity, human-relevant NAMs (iPSC-derived hepatic organoids, liver-on-chip platforms, and complex metabolic MPS), moving toward a research paradigm that is more accurate, ethically sound, and fully aligned with the ASPA 1986 mandate to transition away from regulated animal procedures.

### Compliance Dashboard

| Focus Area | Primary Animal Model | NAM Replacement Technology | ASPA 1986 Status |
|---|---|---|---|
| Metabolic & Hepatic Models | Murine DIO / High-Fat models | Hepatic organoids & Liver-on-chip | High |

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

### Maturity Matrix

**Phase 1 (Exploratory):** Audit current animal use in metabolic syndrome and liver injury studies.

**Phase 2 (Pilot):** Integrate iPSC-derived hepatocyte culture and liver-on-chip protocols into research pipelines.

**Phase 3 (Integration):** Implement multi-organ MPS to model systemic metabolic flux and inter-organ crosstalk.

**Phase 4 (Standardisation):** Replace 100% of high-severity metabolic and hepatic rodent models with qualified 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 barrier integrity and infectious disease models to the complex metabolic and hepatic systems explored in this section (5.3)—demonstrate that replacement is not a siloed scientific exercise but an integrated institutional strategy. By mapping these specialised NAM-based metabolic systems 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

### Strategic Roadmap for NAM Implementation

This matrix provides a phased framework for transitioning from traditional animal-based research to human-relevant, animal-free methods, fulfilling the statutory replacement obligations under the Animals (Scientific Procedures) Act 1986.

| Phase | Maturity Level | Focus Area | Key NAM Integration & Milestone |
|---|---|---|---|
| **Phase 1** | Exploratory | Baseline Assessment | Audit of current animal-use procedures; calibration of iPSC/ primary cell lines against legacy animal data. |
| **Phase 2** | Pilot | Technical Validation | Integration of organoids (gut/lung/brain) into antigen screening and host-pathogen interaction studies. |
| **Phase 3** | Integration | Operational Capability | Deployment of Multi-Organ MPS (Microphysiological Systems) and Lymphoid-on-Chip platforms. |
| **Phase 4** | Standardisation | Full Replacement | 100% replacement of high-severity infection protocols with advanced digital twin modeling and systemic immune-kinetic systems. |

## Implementation & Compliance Framework

**Purpose:** This matrix is designed for Principal Investigators and AWERB to identify capability gaps, prioritise capital investment, and transition research pipelines toward human-relevant models.

**Weight-of-Evidence (WoE) Approach:** Institutional progress must be supported by a robust WoE dossier. Researchers should aggregate data from *in silico* modelling, transcriptomic integration, and MPS-derived kinetics to justify the cessation of regulated animal procedures.

**Regulatory Alignment:** Consistent application of this framework ensures research outputs align with Home Office/ASRU expectations for non-animal method adoption, streamlining license approvals and reporting.

**Strategic Note:** This roadmap is a living instrument. Teams should utilise the "Compliance Dashboard" to track progress against these milestones, ensuring the institution maintains a future-proof, human-relevant research infrastructure.