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

Immunology / Lymphoid Models

Reference 2.1 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 for immunology and lymphoid research (particularly rodent models for immunotherapy, autoimmune disease, vaccine response, immune-oncology, and inflammatory disease) have significant limitations. These systems fail to accurately mirror human immunological phenotypes due to fundamental biological disparities. Key disparities include:

- **Lymphoid Architecture & Complexity:** Human lymphoid tissue—including organised lymph nodes, thymus, and spleen—possesses structural and spatial complexity that is poorly recapitulated in murine models, fundamentally altering immune cell maturation and activation pathways.
- **Immune Cell Lineage & Receptor Repertoire:** Differences in T-cell and B-cell receptor repertoires, combined with distinct cytokine network dynamics, mean that human immune responses are frequently masked or misrepresented in animal-based assays.
- **Immune–Tissue Crosstalk:** The integrated communication between immune cells and specialised tissue stroma in humans involves signalling networks that do not translate reliably to rodent models, often leading to failure in predicting clinical efficacy or off-target toxicity.
- **Severity:** Procedures required to "humanise" rodent immune systems—such as systemic irradiation and invasive engraftment—inflict high-severity systemic stress, organ damage, and significant animal suffering.

Accelerating the adoption of human-relevant Non-Animal Methodologies (NAMs)—such as lymph node-on-chip, thymus/bone marrow organoids, and multi-organ immune MPS—is therefore both a scientific imperative (better prediction of human immune responses and improved translational success) and a legal imperative for UK universities.

Landmark Regulatory & Scientific Momentum

Significant progress has been made in the development and application of human immunology and lymphoid NAMs:

• **Lymph Node-on-Chip / Lymphoid Organoids & MPS** faithfully recapitulate T-cell and B-cell zones, germinal centre formation, dendritic cell–T-cell interactions, cytokine gradients, and immune cell trafficking. They are used for immunotherapy development, vaccine response studies, autoimmune disease modeling, and checkpoint inhibitor testing with high human relevance.

• **Thymus and Thymic Organoids / Thymic Epithelial Cell Models** from human iPSCs or primary cells model T-cell development, positive/negative selection, and thymic microenvironment. They are applied to study immunodeficiency, autoimmune disease mechanisms, and T-cell therapy development.

• **Spleen and Splenic Organoids / MPS and Bone Marrow and Haematopoietic Stem Cell Organoids** / MPS enable study of splenic immune function, haematopoiesis, stem cell niches, and immune cell production in human-relevant 3D systems.

• **Advanced Immune Organoids & Multi-Cellular Lymphoid Assembloids** incorporating stromal, myeloid, and lymphoid components provide complex models of immune tissue architecture and function for inflammatory disease and immune-oncology research.

• **Patient-Derived iPSC Immune / Lymphoid Disease Models** enable precision modelling of genetic immunodeficiencies, autoimmune diseases, and immune cell dysfunction for personalised therapy development.

• **Multi-Organ Immune / Inflammatory MPS** (including gut–immune, lung–immune, joint–immune, and brain–immune extensions) enable holistic study of systemic immune responses, inflammatory crosstalk, and immune–tissue interactions as direct non-animal platforms.

These advancements position lymph node-on-chip/lymphoid organoids, thymic and bone marrow models, advanced immune assembloids, and multi-organ immune MPS as powerful, increasingly accepted alternatives for immunology, immunotherapy, and inflammatory disease research.

Core Specialised Immunology / Lymphoid Models & Enabling platforms

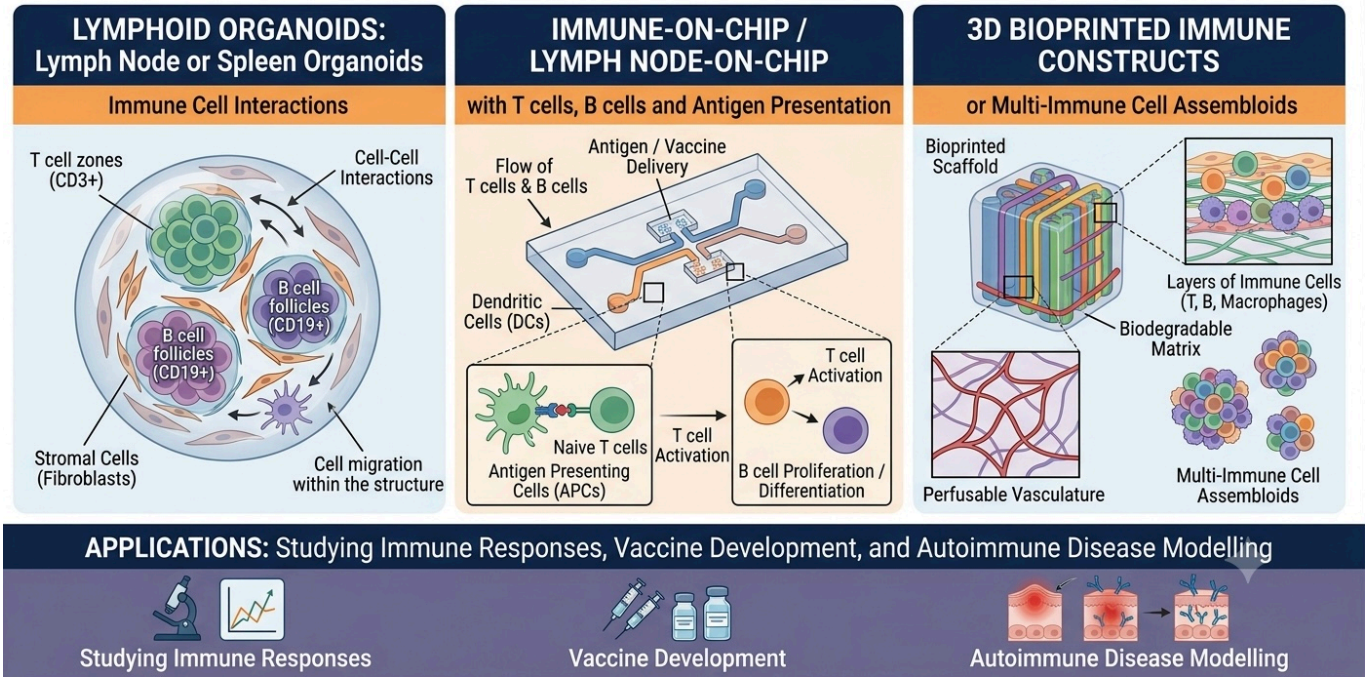

Figure 1: Core Specialised Immunology / Lymphoid Models — Lymphoid organoids (lymph node or spleen organoids) with immune cell interactions (left), immune-on-chip / lymph node-on-chip with T cells, B cells and antigen presentation (center), and 3D bioprinted immune constructs or multi-immune cell assembloids (right) for studying immune responses, vaccine development, and autoimmune disease modelling.

TABLE 1: Core Specialised Immunology / Lymphoid Models

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
Lymph Node, Thymus & Spleen Organoids / MPS	Microfluidic platforms, primary/ iPSC-derived 3D models, and bio-printed constructs recapitulating distinct immune zones (T/B-cell niches, germinal centres), thymic epithelium, and splenic architecture (filtration, antigen presentation).	Immunotherapy development, vaccine response studies, autoimmune disease modeling, positive/negative selection mechanisms, and checkpoint inhibitor testing.	High human relevance for immune tissue architecture and systemic immunity; enables T-cell therapy training without animal models.
Bone Marrow & Advanced Multi-Cellular Assembloids	Advanced 3D organoids incorporating stromal, myeloid, and lymphoid components to recreate hematopoietic stem cell (HSC) niches and complex immune microenvironments.	Haematopoiesis, stem cell biology, immune cell production, complex immuno-oncology modeling, and inflammatory disease tissue function.	Enables human HSC/ haematopoiesis research; excellent training ground for advanced immuno-oncology and inflammatory disease modelling.

Cross-Cutting & Enabling Platforms for Immunology / Lymphoid Research

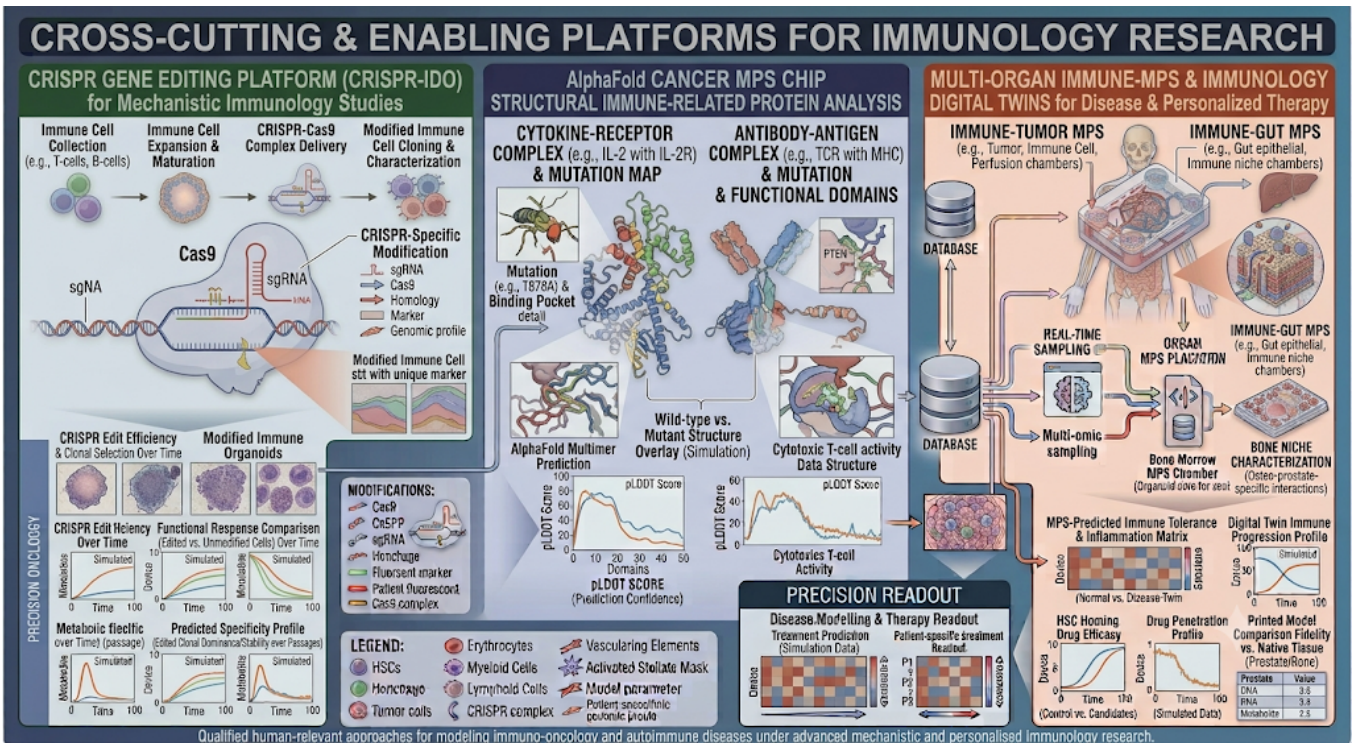

Figure 2: Cross-Cutting & Enabling Platforms for Immunology Research — CRISPR gene editing in lymphoid or immune organoids (left), AlphaFold predicting structures of immune-related proteins such as cytokines and receptors (center), and multi-organ immune–tumor or immune–gut MPS plus digital twins for immuno-oncology and autoimmune disease modelling (right) for advanced mechanistic and personalised immunology research.

Table 2: Cross cutting & Enabling Platforms in Immunology / Lymphoid Research

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
Patient-Derived iPSC Immune Models & CRISPR Integration	Reprogrammed patient iPSCs differentiated into immune lineages, assembled into lymphoid organoids, and paired with CRISPR-based functional genomics to model or correct inherited/acquired immune disorders.	Precision mechanistic studies, gene-editing correction, target validation, regulatory network tracking, and personalized immunotherapy development.	Directly addresses animal model genetic limitations; powerful human-relevant tool for mutation-specific immunology and genetic replacement arguments.
Multi-Organ Immune MPS, AlphaFold & Digital Twin Integration	Interconnected microphysiological systems linking lymphoid/immune compartments with gut, lung, joint, brain, or tumor tissues - fully integrated with AlphaFold protein modeling and predictive computational digital twins.	Systemic immune responses, inflammatory tissue crosstalk, predictive modeling of immunotherapy efficacy, molecular target structural modeling, and multi-organ toxicity	Holistic systems approach; ideal for translational systems immunology training and robust computational/ predictive replacement frameworks.
AOP-Integrated NAMs for Immunotoxicity & Modulation	Adverse Outcome Pathway (AOP) frameworks combining in vitro, omics, and computational weight-of-evidence data to track cellular stress cascades to regulatory endpoints.	Immunotoxicity assessment, safety screening, immune modulation prediction, and regulatory weight-of-evidence dossier compilation.	Supports regulatory science, safety assessment, and compliance training aligned with modern regulatory expectations.

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 Immunology/Lymphoid & Data Science:** Training programmes should bridge the gap between bench-top laboratory biology and computational analysis. Researchers require dual proficiency in 3D culture techniques and the digital pipelines used to interpret complex imaging and high-throughput drug-response 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 CRISPR gene editing, organoid culture, AlphaFold/AI-driven protein structure prediction, multi-organ microphysiological systems (MPS), and digital twin modelling are increasingly critical for modern immunology and lymphoid 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	Lymphoid organoid & chip engineering	Direct Replacement of murine models
Bioinformatics	Immune-transcriptome data analysis	Bypasses animal-based tissue sampling
Regulatory	ASPA 1986 Compliance & AWERB	Facilitates faster ASRU approvals
Computational	Multi-organ immune-metabolic modelling	Eliminates invasive animal efficacy 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: CRISPR-Edited Immune Cells in Lymphoid Models Reaching Clinical Trials

- **Background:** Traditional development of advanced cell therapies often relies heavily on animal models that poorly predict human immune responses, particularly in complex lymphoid and tumour microenvironments.
- **The Paradigm Shift:** CRISPR gene editing combined with lymphoid organoids and immune-on-chip systems has accelerated the path to clinic. Multiple CRISPR-engineered T-cell and CAR-T therapies, validated in human immune organoid models, have progressed into active clinical trials for haematological malignancies, solid tumours, and autoimmune diseases.
- **The Methodology:** Researchers use CRISPR to precisely modify genes in primary immune cells or within patient-derived lymphoid organoids. These edited cells are then tested in advanced microphysiological systems (MPS) that recreate lymph node-like structures, immune–tumour interactions, or multi-organ immune responses. This human-relevant data supports IND submissions and refines dosing and safety 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 (Replacement, Reduction, Refinement) and ASPA 1986 requirements.

Case Study 2: AlphaFold-Enabled Immune Protein Therapies Entering First-in-Human Trials

AI-driven protein structure prediction tools such as AlphaFold are now directly contributing to clinical candidates. Companies like Isomorphic Labs have advanced AlphaFold-designed molecules targeting immune-related proteins (cytokines, receptors, and checkpoints) into early-phase oncology and immunology clinical trials (2025–2026). These candidates were optimised using structural insights that would have taken years to obtain experimentally.

Case Study 3: Multi-Organ Immune MPS and Digital Twins in Immuno-Oncology and Autoimmune Trials

Pharmaceutical and biotech companies are increasingly incorporating multi-organ immune–tumour or immune–gut 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 rheumatoid arthritis, psoriasis, inflammatory bowel disease, and immuno-oncology to reduce patient numbers, enhance statistical power, and enable precision dosing.

Executive Summary & Compliance Dashboard

Executive Summary

Traditional rodent models for immunology often fail to capture the complex, human-specific immune responses and lymphoid tissue architecture. This roadmap transitions research toward human-relevant Immunology / Lymphoid NAMs (New Approach Methodologies). By adopting these high-fidelity systems, the institution enhances scientific reproducibility, reduces dependency on unreliable cross-species extrapolation, and fulfills the ASPA 1986 mandate.

Compliance Dashboard

Focus Area	Primary Animal Model	NAM Replacement Technology	ASPA 1986 Status
Immunology / Lymphoid	Primate/Rodent Immunisation & Disease Models	Organoids (e.g., Lymph Node, Spleen), Lymphoid-on-Chip, Humanised Mice (as a step), In Silico Modelling	High

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

Maturity Matrix

Phase 1 (Exploratory): Audit current primate and high-severity rodent immune model use for safety and efficacy.

Phase 2 (Pilot): Integrate human lymphoid tissue organoids and lymph node models into antigen stimulation and vaccination screening.

Phase 3 (Integration): Humanication via implementation of complex multi-organ lymphoid-on-chip systems and humanised mice with validated immune cell humanisation.

Phase 4 (Standardisation): Replace 100% of high-severity primate and rodent immunisation protocols for safety testing.

Final Compliance Statement Conclusion

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 and immunology research.

Integration Context: Roadmap Conclusion

The modules detailed in this roadmap—from fundamental oncology (1.1) and haematology (1.2) to breast cancer (1.3), prostate cancer (1.4), and immunology / lymphoid models (1.5)—demonstrate that replacement is not a siloed scientific exercise but an integrated institutional strategy. By mapping these specialised NAMs into a unified framework, the institution moves 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 defines the developmental path toward full replacement of animal-based immunology models, in alignment with the statutory duties of the Animals (Scientific Procedures) Act 1986.

Phase	Focus Area	Key NAM Integration
Phase 1	Exploratory	Baseline assessment; calibration of human-derived cell lines against legacy animal data.
Phase 2	Pilot	Integration of human lymphoid organoids and lymph node models into antigen screening.
Phase 3	Integration	Implementation of complex multi-organ lymphoid-on-chip systems; systemic immune distribution modelling.
Phase 4	Standardisation	100% replacement of high-severity immunisation protocols with advanced computational (AI/ML) & multi-organ systems.

Implementation Guidance:

Purpose: This matrix is a self-assessment and planning tool for Principal Investigators and AWERB members to identify capability gaps and prioritise investment.

Progression: Stages are iterative. Teams should use this to identify which dimensions of their research (e.g., cell lineage modelling, tissue crosstalk) require the most urgent advancement.

Evidence Base: Progress through these phases should be supported by a "Weight-of-Evidence" (WoE) dossier, aggregating data from *in silico*, *in vitro*, and *ex vivo* human models to justify the move away from regulated procedures.